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UBASH3A and TIGIT genes expression levels in systemic sclerosis

Aml A. R. Mohammed[1], Fatma S. Abd-Elsamea[2], Maha S. I. Abdelrahman[3], Fatma M. A. Mohamed[4], Fatma Y. A. Abbas[5], and Fatma M. Helbawi[1]

[1]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[2]Department of Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[3]Department of Rheumatology, Rehabilitation & Physical Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt.

[4]Department of Histology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[5]Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Fatma S. Abd-Elsamea, Department of Microbiology & Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.
Email: fatmasayed@aun.edu.eg.

Abstract

Systemic sclerosis (SSc) is an autoimmune disorder marked by excessive fibrosis, microvascular stenosis, and systemic clinical manifestations. An autoimmune process is believed to induce T-cell activation, mainly CD 4 T helper cells, and enhance production of proinflammatory and profibrotic cytokines such as interleukin (IL) 4 and IL 13. These cytokines contribute to vasculopathy and excessive collagen synthesis. UBASH3a (Ubiquitin Associated and SH3 Domain Containing A) and TIGIT (T cell immunoglobulin and ITIM domain) have an important role in limiting unwarranted T cell activation and maintaining immune tolerance. Our study aimed to explore UBASH3A and TIGIT mRNA expression levels in 30 SSc patients compared to 30 age and sex matched normal controls. We measured mRNA levels via real-time quantitative reverse transcription polymerase chain reaction in total RNA isolated from the peripheral blood mononuclear cells (PBMCs) of SSc patients and controls. We used RNA extraction commercial kits. The expression levels of UBASH3A and TIGIT mRNA were significantly higher in PBMCs from SSc patients compared to control subjects.

Keywords: UBASH3A, TIGIT, T cell, Systemic Sclerosis

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Introduction

Systemic sclerosis (SSc) is an autoimmune disease, which is characterized by skin tightness and systemic manifestations. Environmental and genetic factors are likely to be implicated in SSc etiology.[1] Pathogenesis includes evidence of autoimmunity with distinct autoantibodies, activation of innate and adaptive immunity and vasculopathy leading to skin and organ fibrosis. This fibrosis causes irreversible organ damage which contributes to high morbidity and mortality.[2] Cases with significant pulmonary or cardiac involvement have a three-year survival rate of 47-56%.[3] In SSc, T cells were proven to have a significant role. They infiltrate tissues very early in the disease course contributing to inflammation and fibrosis.[4]

Ubiquitin Associated and SH3 Domain Containing A (UBASH3A) is a type of protein

tyrosine phosphatase family that is involved in the regulation of T cell receptor signaling. It is mainly implicated in tyrosine phosphorylation regulation within T cells and the negative regulation of T-cell signaling.[5] UBASH3A deficiency eases T-cell response to T-cell receptor (TCR)/CD3 complex stimulation, consequently aggravating T-cell-dependent inflammation.[6] It enhances T cells apoptosis via binding to the apoptosis-inducing protein apoptosis-inducing factor, a key factor of caspase-independent apoptosis.[7]

Genetic polymorphisms and altered expression levels of UBASH3A were associated with multiple autoimmune diseases (ADs), such as systemic lupus erythematosus (SLE),[8] and rheumatoid arthritis (RA).[9]

Co-inhibitory receptors have a crucial role in preserving immune homeostasis via regulation of T cell function. Among these, T cell immunoglobulin and ITIM domain (TIGIT) has emerged as a key regulator of T cell function. TIGIT, expressed on various immune cells including T cells and natural killer cells, interacts with its ligands, CD155 and CD112, leading to the suppression of immune responses. Studies have shown an increased expression of TIGIT in lymphocytes from SSc patients, indicating that altered regulatory capacity of co-inhibitory receptors may contribute to disease pathophysiology.[10, 11]

Given the involvement of both UBASH3A and TIGIT in immune regulation, investigating their expression in SSc may provide valuable insights into disease mechanisms and potential therapeutic targets.

Subjects and Methods

This hospital-based cross-sectional case-control study included 30 SSc patients and 30 normal volunteer controls. Patients were recruited from the settings of the Department of Rheumatology, Rehabilitation, and Physical Medicine in tertiary care hospital during the period between March 2023 and October 2023.

Patients were included in the study if they were older than 18 years of age and had confirmed SSc diagnosis according to the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) classification criteria of SSc.[12] Patients were excluded if they had evidence of other rheumatological disease or malignancy.

Complete history taking, clinical assessment, and routine laboratory investigations were done. Clinical assessment included:

- Modified Rodnan skin score

This was used to assess skin thickness, graded by clinical palpation from 0 to 3. High scores represented more severe skin tightness. It included examinations of 17 surface anatomic areas; face, anterior chest, abdomen, (right and left) fingers, forearms, upper arms, thighs, lower legs, dorsum of hands and feet. Separate values were summed to get the total skin score.[13, 14]

- European Scleroderma Study Group (EScSG) activity index

This consisted of 10 items. Each item has a constant numerical value from 0.5 - 2.0. The total score ranged from 0 to 10. The items included Modified Rodnan skin score > 14, scleredema, changes in skin stiffness within one-month, digital necrosis, vascular symptoms changes within one month, arthritis, diffusion lung capacity < 80%, cardiopulmonary symptoms changes, erythrocyte sedimentation rate >30mm/1.hour and hypo-complementemia.[15-17]

Patients underwent laboratory testing to assess hematological, inflammatory, and immunological parameters relevant to systemic sclerosis. Complete blood count (CBC) analysis was performed via an automated machine (ADVIA 2120i hematology system, Siemens, Germany). The Neutrophils to Lymphocyte ratio (NLR) was determined by dividing the absolute neutrophil count by the absolute lymphocyte count. Erythrocyte sedimentation rate (ESR) was measured on an automated analyzer (Alifax Roller 20LC Fully Automated ESR Analyzer, Alifax S.r.l., Polverara, Italy). C-reactive protein (CRP) and Complement components C3 and C4 levels were analyzed on an automated nephelometric analyzer (BN ProSpec analyzer, Siemens, Germany). Antinuclear antibody (ANA) testing was also assessed by indirect

immunofluorescence technique using commercial kits (HEP 2 cells, Diasorin ANA Fluoro, REF 1660, Diasorin Inc, Germany), according to the manufacturer's instructions.

Peripheral blood mononuclear cells (PBMCs) were isolated from freshly drawn EDTA blood by Ficoll-Paque gradient centrifugation using lymphocyte separation media (Lymphosep supplied by Biowest, France), according to the manufacturer's protocol. The PBMCs were stored at −80°C until used.

Total RNA was extracted from the PBMCs of the two study groups using an RNA extraction kit (Cat.NO. K0731, GeneJET RNA Purification Kit, Thermo Fisher Scientific Co; USA). RNA concentration was assessed using a nanodrop spectrophotometer (BioTek Epoch NanoDrop, USA). The RNA was reverse transcribed into complementary DNA (cDNA) using High-Capacity cDNA Reverse Transcription Kit (Cat. NO. 4368813, Thermo Fisher Scientific Co; USA), according to manufacturer's protocol.

The real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was performed in a 20 µl volume that contained 10 µl of SYBR Premix Ex Taq II, 0.1 µg of cDNA template, 1 µl of forward primers (final concentration 0.4 µmol/l), and 1 µl of reverse primers (final concentration 0.4 µmol/l). Relative expressions were normalized to β-actin for endogenous control. △Ct was recorded automatically and relative expression levels of both genes were calculated by the 2−△△Ct method.

UBASH3A and TIGIT gene primers were designed from the gene bank (NCBI) mRNA sequences, targeting shared regions across gene variants. The forward (F) and reverse (R) PCR primers used were as follows:

UBASH3A (NC_000021.9):

F: 5'-TTG GCC CAC AAG TTC TAC CC -3'
R: 5'-CGT CAG CTC ATC CAC GTT CT -3'

TIGIT (NM_173799.4):

F: 5'-GGA GGT CCT AGA AAG CTC AGT G- 3'
R: 5'-CGA TGA CTG CTG TGC AGA TGA- 3'

As an internal control, the β-actin gene was utilized, and its primer sequence was as previously published.[18]

F: 5'-ATC TGG CAC CAC ACC TTC TAC AAT GAG CTG CG- 3'
R: 5'-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC- 3'

The fold changes (FCs) of the target mRNAs were normalized using the β-ACTIN, and then, the FCs of each mRNA were calculated based on the ratio between the patient and control groups. The experiment was repeated three times (in triplicate) to confirm the results. The threshold cycle value (2−ΔCT) was used for statistical analysis, and the results are presented as FCs.

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20. Categorical data are presented as frequencies and percentages. Numerical data were checked for normality by Shapiro-Walk test and are presented as mean and standard deviation (SD) or median and range. The Mann-Whitney U test was applied to compare the median difference of the FCs of UBASH3A and TIGIT between patients and controls. Spearman's correlation was utilized to identify the correlation between the FCs of both UBASH3A and TIGIT and different variables.

Results

The demographic features of the study patients are presented in Table 1. No statistically significant difference in age, gender, and other demographic data was observed between SSc patients and controls.

Clinical manifestations and the laboratory investigations of patients with SSc are presented in Table 2 and 3, respectively.

Table 1. Sociodemographic data of the 30 systemic sclerosis patients.

Variables	Number	%
Age (years) Mean ±SD (Range)	41.77±9.73 (18.0-60.0)	
Gender		
Male	4	13.3%
Female	26	86.7%
Marital status		
Married	23	76.7%
Single/widow/divorced	7	13.3%
Smoking		
Smoker	1	3.3%
Passive smoker	17	56.7%
Non-smoker	12	40.0%
Family history of rheumatological disease	6	20.0%
Anthropometric measures		
Height (cm) Mean ±SD	162.57±5.60	
Weight (kg) Mean ±SD	65.13±14.05	
BMI Mean ±SD	24.53±4.58	

BMI: Body Mass Index. Data are presented as mean± SD (range) or frequency (%).

Table 2. Clinical manifestations of the 30 systemic sclerosis patients.

Variables	Number	(%)
Disease duration in years: Median (range)	7.0 (1.5-21.0)	
Age at disease onset in years: Median (range)	35.50 (7-55)	
Musculoskeletal manifestations		
Arthralgia	17	56.7%
Arthritis	2	6.7%
Myalgia	2	6.7%
Vascular and mucocutaneous manifestations		
Raynaud's Phenomenon	30	100.0%
Digital scar	27	90.0%
Telangiectasia	10	33.3%
Salt and pepper appearance	9	30.0%
Digital ulcer	7	23.3%
Dry eye	10	33.3%
Dry mouth	8	26.7%

Table 2. Continued.

Variables	Number	%
Chest and Cardiovascular manifestations		
IPF	22	73.3%
PASP (mmHg): Mean ± SD (range)	28.30±8.69 (12-57)	
Modified Rodnan skin score: Mean ± SD (range)	17.83±5.86 (9-34)	
Disease activity index: Mean ± SD (range)	3.13±1.56 (0.5-7.0)	
Disease subsets		
Limited	21	70.0%
Diffuse	9	30.0%

IPF: interstitial pulmonary fibrosis; PASP: pulmonary artery systolic pressure.
Data are presented as mean ± SD/ median(range) or frequency (%)

Table 3. Laboratory investigations among the 30 systemic sclerosis patients.

Parameter	Results
CBC	
WBCs (x 10^6/L): Mean ± SD	5.89±1.89
Neutrophils%: Mean ± SD	57.84±12.73
Neutrophils absolute (x 10^6/L): Mean ± SD	3.49±1.74
Lymphocytes %: Mean ± SD	29.79±11.05
Lymphocytes absolute (x 10^6/L): Mean ± SD	1.66±0.72
NLR: median (range)	1.6 (0.68-10.37)
RBCs (x 10^9/L): Mean ± SD	4.35±0.61
Hemoglobin (g/dl): Mean ± SD	12.08±1.54
Platelets (x 10^6/L): Mean ± SD	272.20±85.97
Inflammatory markers	
ESR (mm/hr): median (range)	35.0 (3.0-107)
CRP (mg/l): median (range)	4.60 (0-22.0)
Immunological markers	
C 3 (mg/dl)	124.89±25.17
C 4 (mg/dl)	25.73±7.44
ANA	
Positive	28 (93.3%)
Negative	2 (6.7%)

CBC; complete blood count, WBC: white blood cells, NLR: Neutrophils/ Lymphocyte ratio, RBCs: Red blood cells, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, C3: Complement 3, C4: Complement 4, ANA: Antinuclear antibody.

We observed a statistically significant higher median FC expression of UBASH3A in patients with SSc (median 2.31, range: 0.53-19.55) compared to controls (1.36, 0.01-7.25), p= 0.011 as illustrated in Figure 1. In addition, patients with SSc exhibited a statistically significantly higher median FC expression of TIGIT compared to healthy controls (median 3, range 2.4-26 vs 1.45, 0.07-2.7), p< 0.001, as illustrated in Figure 2.

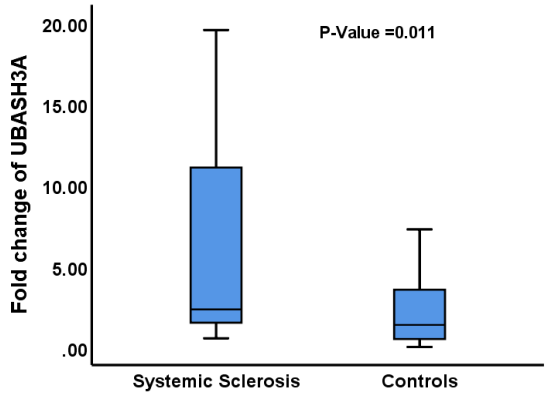

Figure 1. Boxplot for the distribution of Fold change of UBASH3A among systemic sclerosis patients and controls.

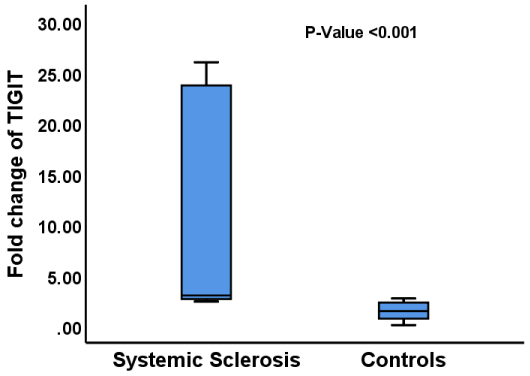

Figure 2. Boxplot for the distribution of Fold change of TIGIT among systemic sclerosis patients and Controls.

Table 4 shows a statistically significant moderate positive correlation between the FC expression of UBASH3A and TIGIT in patients with SSc (r=0.596, *p*=0.001). Moreover, there was a statistically significant moderate positive correlation between the FC of TIGIT and CRP level (r=0.415, *p*=0.022). However, no statistically significant correlation was found between the FCs of TIGIT or UBASH3A and age, disease duration, age of disease onset, modified Rodnan skin score, EScSG disease activity index, PASP, and other laboratory investigations.

Table 4. Correlation between Fold changes of UBASH3A and TIGIT and various variables among systemic sclerosis patients.

Variables	Fold change of UBASH3A gene		Fold change of TIGIT gene	
	r	*p*-value*	r	*p*-value*
Fold change of TIGIT gene	0.596	0.001		
Age	0.214	NS	0.074	NS
Disease duration in years	0.009	NS	0.051	NS
Age of disease onset	0.207	NS	0.074	NS
Modified Rodnan skin score	0.191	NS	0.306	NS
Disease activity index	-0.152	NS	0.166	NS
PASP	0.262	NS	0.030	NS
Laboratory Investigations				
WBC (x 10^6/L)	0.096	NS	0.075	NS
Neutrophils %	0.142	NS	0.110	NS
Neutrophils absolute (x 10^6/L)	0.145	NS	0.157	NS
Lymphocytes %	-0.088	NS	-0.074	NS
Lymphocytes absolute (x 10^6/L)	0.017	NS	0.012	NS
NLR	0.105	NS	0.094	NS
RBCs (x 10^9/L) _	0.057	NS	-0.116	NS
Hemoglobin (g/dl)	0.021	NS	-0.131	NS
Platelets (x 10^6/L)	0.056	NS	0.082	NS
ESR (mm/hr)	0.052	NS	0.168	NS
CRP (mg/l)	0.178	NS	0.415	0.022

*Spearman correlation, R (correlation coefficient), PASP: pulmonary artery systolic pressure, WBC: White blood cells, NLR: Neutrophils/ Lymphocyte ratio, RBCs: Red blood cells, ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein.
p > 0.05 is not significant (NS).

Table 5 shows a statistically significant higher median expression of the FC of TIGIT among patients with the diffuse SSc disease in comparison to the limited subtype (p=0.007). However, there was no statistically significant difference in the expression of FC of UBASH3A between limited and diffuse SSc disease subsets.

Table 5. Association between Fold changes of both UBASH3A and TIGIT and systemic sclerosis disease subtypes.

Variables	Fold change of UBASH3A	Fold change of TIGIT
Type of SSc Disease		
Limited	1.70 (0.90-19.55)	2.79 (2.40-26.00)
Diffuse	2.53 (0.53-19.53)	24.00 (2.60-25.00)
p-Value*	NS	0.007

Data is expressed as median (range); *Mann Whitney U test. $p > 0.05$ is not significant (NS).

Discussion

UBASH3A and TGIT play an important role in limiting unwarranted T cell activation and maintaining immune tolerance, thus they assume a significant role in unraveling SSc pathogenesis.[10] Our study analyzed the expression levels of both UBASH3A and TIGIT genes in SSc patients in comparison to normal control subjects and correlated these expression levels with the clinical and laboratory data of the studied patients.

Genetic variants in UBASH3A were associated with various ADs such as type 1 diabetes[19] and RA.[20] Previous studies linked UBASH3A role in T cells to its contribution to the preservation of immunological homeostasis, which protects against autoimmune and chronic inflammatory conditions. The major influence of UBASH3A on T cells may be related to its role in apoptosis-inducing factor -mediated apoptosis. UBASH3A deletion may increase the persistence of activated T cells.[21]

To the best of our knowledge, this study is the first one that investigated UBASH3A mRNA expression levels in SSc. However, there was no statistically significant difference between limited and diffuse SSc regarding the expression of FC of UBASH3A. In contrast to a previous study on SLE patients,[22] our study detected a statistically significant elevation of UBASH3A expression among patients with SSc compared to controls. This difference may reflect disease-specific immune regulatory mechanisms or a compensatory response to chronic inflammation in SSc.[11]

UBASH3A mRNA expression levels are remarkably decreased in SLE patients in comparison to healthy subjects (p= 0.002). Moreover, disease activity in SLE patients was negatively correlated with UBASH3A mRNA expression levels (p=0.049).[22] This points to the potential protective role of UBASH3A in SLE.

We detected a statistically significant higher median FC expression of TIGIT among patients with SSc compared to controls. In addition, a statistically significant higher median FC expression of TIGIT among patients with diffuse SSc, compared to the limited subtype, was detected. These results match those reported by Fleury et al., 2018, that TIGIT as Co-IR was highly expressed mainly in T cells and natural killer cells in SSc patients in comparison to normal controls.[11] Also, CD4+ T cells and Treg cells from SSc patients had an elevated level of programmed cell death 1 receptor (PD-1) +TIGIT+ double-positive cells compared to those from controls. Exhausted T cells from overwhelming autoimmune processes are characterized by high expression of multiple Co-IRs on the cell surface.[11] So, elevated TIGIT expression suggests a compensatory

mechanism to counteract excessive immune activation. Its higher expression in diffuse SSc aligns with its potential role in disease severity and immune exhaustion.

Increased TIGIT expressing CD4+ T cells has been reported in other ADs. SLE patients exhibited significantly higher TIGIT expression on CD4+ T cells, specifically in cases with high levels of anti-double stranded DNA and anti-Smith antibodies, and proteinuria.[23] Another study detected the upregulation of TIGIT on T cells in RA patients.[24] CD4+ TIGIT+ T cells levels in synovial fluid are negatively correlated to the disease activity in RA patients.[25]

A study conducted by Kurita et al., 2019, revealed that TIGIT expressing CD4+ T cells are increased in mild atopic dermatitis compared with healthy subjects, but a significant reduction in TIGIT+ CD4+ T cells numbers was observed in severe atopic dermatitis cases. They suggested that disease severity may be attributed to a reduction in TIGIT+ CD4+ T cells due to immune exhaustion.[26]

In type 1 diabetes, TIGIT+ Tregs have been shown to be increased.[27] Tregs play a role in controlling autoimmune thyroiditis, as evidenced by the association between the expression of an inhibitory receptor Fc Receptor-Like 3 (FCRL3) and TIGIT in various subsets of the disease.[28]

On the other hand, multiple sclerosis (MS) patients showed lower TIGIT-expressing CD4 cells in comparison to healthy controls ($p< 0.02$).[29] This suggests that TIGIT-expressing Tregs are involved in the suppression of MS.[27]

We observed a statistically significant moderate positive correlation between TIGIT expression and CRP level. This finding is consistent with previous research showing a positive correlation between the levels of TIGIT+ on CD4+ and CD8+ T cells and CRP levels in RA patients.[25] This relationship between TIGIT and CRP, as inflammatory predictor, may indicate a potential link between immune activation and inflammation in SSc.

We detected a statistically significant moderate positive correlation between the expression of FCs of TIGIT and UBASH3A among patients with SSc. This finding may indicate a coordinated role in immune regulation, possibly contributing to T-cell exhaustion and fibrosis progression.

In conclusion, this study highlights the increased expression of UBASH3A and TIGIT in SSc, reinforcing their potential role in disease pathogenesis. These findings imply that immune checkpoint pathways may be implicated in the fibrotic and inflammatory processes underlying SSc, warranting further investigation into their therapeutic implications.

Author Contributions

All authors participated in drafting the article and reviewing it critically for important intellectual aspects, and all authors approved the final version to be published. AARM and FMH had full access to all data in the study and have responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design by FSA and MSIA. Acquisition of data by FYAA and MSIA. Finally, analysis and interpretation of data by FMAM.

Declaration of Conflicting Interests

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Ethical approval

The study was approved by the Institutional Review Board of the Faculty of Medicine, Assiut University (approval No. 04-2023-300172).

Informed consent

A signed consent form was obtained from each study subject.

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