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ORIGINAL ARTICLE

The Role of T-cell Immunoglobulin and Mucin Domain-Containing Molecule-3 as Disease Activity Marker in Systemic Lupus Erythematosus

Lobna Ismaeil kotb[1], Nagwa Ahmad Sherby, Abdelmonaem Mohamed Zoghdani [2], Marwa Aboshabana Moustafa[3*], Mona Rabie[1*]
[1] Rheumatology & Rehabilitation Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt
[2] Rheumatology & Rehabilitation Department, Faculty of Medicine, Tripoli University, Libya.
[3] Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.

***Correspondence author:**
Marwa Aboshabana Moustafa and Mona Rabie

Email:
MRMustafa@medicine.zu.edu.eg
mona_22785@yahoo.com

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is an example of a systemic autoimmune illness. Although the T cell immunoglobulin and mucin domain (TIM) family is linked to autoimmune disorders, it is yet unknown how much of this family is expressed in the immune cells of SLE patients. We hypothesized to detect if serum human T cell immunoglobulin mucin 3 (sTIM-3) level is elevated in lupus patients and its correlation with the activity of the disease.

Methods: This study was conducted on 112 individuals diagnosed with SLE who were receiving treatment at the Rheumatology and Rehabilitation Department at Zagazig University Hospitals. The serum level of sTIM-3 was measured using the TIM-3 ELISA Kit (Enzyme-Linked Immunosorbent Assay). Patients' activity was assessed by the SLEDAI-2K score. Activity categories were defined based on SLEDAI grades.

Results: Statistically significant differences were detected between different SLEDAI-2K grades and sTIM-3 levels, as patients with moderate to very high disease activity showed the highest sTIM-3 levels.

Conclusion: sTIM-3 levels were correlated with the activity of SLE disease. These results suggested a close relationship between circulating sTIM-3 and active SLE. However, it is still not valid to discriminate against patients with high activity.

Keywords: T-cell Immunoglobulin, Mucin Domain-Containing Molecule-3, Systemic Lupus Erythematosus, SLEDAI-2K.

Key points:
*There is a strong correlation between the active SLE and the levels of circulating sTIM-3.
* For assessing the level of disease activity, TIM-3 is a sensitive biomarker, but it is still not valid to discriminate SLE patients with high disease activity

INTRODUCTION

Systemic lupus erythematosus (SLE) is known as an autoimmune chronic condition and is defined by the production of immunological complexes in multiple organs, which spread widely and cause multisystem diseases. There is usually involvement of organs encompassing the blood vessels, kidneys, heart, lungs, skin, joints and neurological system. Even though SLE is a cyclical condition, its flare-ups and remissions are unpredictable. Therefore, in order to assess the disease activity, an accurate biomarker must be developed.[1]

According to the most recent findings in immunology research, human T cell immunoglobulin mucin 3 (TIM-3) malfunction impacts many immune cells and contributes to the etiology of illnesses. Receptor-ligand axis activity monitoring can be used as a unique biological marker for prognostic assessment and clinical

diagnosis of disease. More significantly, interventions for the treatment of autoimmune-related disorders may focus on the downstream signaling pathway components and the TIM-3-ligand axis.[2]

Immune checkpoint molecules likely facilitate the interaction and stimulation of immunological cells. TIM-3 is an immune checkpoint molecule that has a role in the suppression of immunological responses. Galactin-9 (Gal-9), a molecule that binds to TIM-3, promotes the improvement of autoimmune disorders.[3]

T-cell immunoglobulin and TIM-3 have a role in a number of chronic autoimmune diseases, like rheumatoid arthritis and multiple sclerosis. It accomplishes this by regulating the immunological responses of T cells and preserving the balance between T helper (Th17) and T regulatory (Treg) cells. In addition, new investigations have discovered strong connections between the emergence of TIM-3 and SLE. The increased TIM-3 messenger RNA (mRNA) levels in peripheral blood mononuclear cells (PBMCs) were initially observed among those suffering from SLE, and there is a connection between Interferon- γ (IFN-ɤ) and TIM-3 expression. The expression of TIM-3 on the membrane of certain peripheral T cells was correlated with the degree of disease activity in people with SLE.[4] So, we aimed to detect if the sTIM-3 level is upregulated in lupus patients and its correlation with the activity of the disease.

METHODS

This cross-sectional study was carried out on 112 individuals diagnosed with SLE recruited between January 2023 and January 2024 from the inpatient and outpatient clinics of the Rheumatology and Rehabilitation Department, Zagazig University Hospitals, after the approval of the Institutional Review Board (ZU-IRB#9771/6-9-2022). A signed consent form was obtained for every patient.

Inclusion criteria included SLE patients older than 18 years. All patients fulfilled the 2019 EULAR/ACR classification criteria for SLE.[5] Exclusion criteria included SLE patients suffering from other autoimmune diseases, a history of infection, or malignancy at the time of study.

Every patient had a thorough medical history taken, and they were subjected to general examination, locomotor, and other systems examination. All laboratory parameters used in SLEDAI-2K were documented. Patients` disease activity was assessed by SLEDAI-2K score.[6] Activity categories determined by SLEDAI grades[7]: SLEDAI = 0 indicates no activity, SLEDAI = 1–5 indicates mild activity, SLEDAI = 6–10 indicates moderate activity, SLEDAI = 11–19 indicates strong activity, and SLEDAI ≥ 20 indicates very high activity.

A human TIM-3 ELISA (Enzyme-Linked Immunosorbent Assay) Kit was used to evaluate the level of sTIM-3 in serum. The test principle for this kit is to determine the quantitative level of human TIM-3 in a sample. The method involves coating microtiter plates with purified TIM-3 antibodies to create solid-phase antibodies. TIM-3 was added to the wells and combined with the TIM-3 antibody labelled in order to create an antibody-antigen-enzyme-antibody complex using horseradish peroxidase (HRP). The plate was then washed, and tetramethylbenzidine (TMB) substrate solution was added. The HRP enzyme catalyzed a reaction that caused the TMB substrate to turn blue. The reaction was stopped by the color shift, which was observed at 450 nm after adding a stop solution. The concentration of TIM-3 in the samples was determined by comparing their optical density to a standard curve.

STATISTICAL ANALYSIS:

The data acquired underwent coding, data entry, presentation, and analysis using a computerized database software application called the Statistical Package for Social Science (SPSS) version 26. Frequencies and percentages were utilized to illustrate the qualitative data. The mean, median, interquartile range (IQR), and standard deviation (SD) were utilized for quantitative variables. To evaluate the link between the variables, Spearman's rank correlation coefficient was computed. The Kruskal-Wallis test was employed to compare more than two variables. The validity of sTIM-3 was determined using receiver operating characteristic (ROC) curve analysis for the detection of the optimal cutoff value of sTIM-3 for high disease activity.

RESULTS:

The majority of the 112 SLE patients in this study were female, with a mean age of 33.43±8.85 years and a mean disease duration of 5.05±4.29 years. The mean SLEDAI-2K score was 7.25 ± 8.03. About 44.6% of cases showed mild to moderate activity grade, and high activity to very high cases were 27.7%. Most of the cases treated with hydroxychloroquine (92.8%) and corticosteroids were taken in 87.5% of cases, **as shown in Table 1.**

The activity measures distribution among patients exhibited significant variability. In 55% of the patients, mucocutaneous and arthritis activity were most common. Conversely, among the 2.5%

and 7.5% of patients, respectively, cardiac involvement and vasculitis were uncommon.17.5% of the patients exhibited hypocomplementemia, a sign of serologic activity, and 37.5% of the patients had a high titer of anti-double-stranded DNA.

Regarding sTIM-3 concentration, the mean serum level was 550.96 ± 204.43 pg/ml, with a median of 481.44 pg/ml, **as illustrated in Figure 1.** There were statistically significant positive correlations between sTIM-3 levels and SLEDAI-2K score and 24-hour urinary protein, while there were no significant correlations of sTIM-3 levels with other laboratory measures, **as illustrated in Table 2.**

A statistically significant difference in sTIM-3 levels was detected between SLE patients with different SLEDAI-2K score grading. sTIM-3 showed higher levels in patients with moderate to very high activity than those with no to mild activity, **as shown in Table 3**.

We employed an ROC curve to assess the validity of sTIM-3 for detecting disease activity in SLE patients. **Figure 2's** ROC curve showed an area under the curve of 0.662 with a 95% confidence interval (CI) of (0.494-0.831). It determined a cutoff value of 458.43 pg/ml for detecting disease activity in SLE patients, with a sensitivity of 73.9% and a specificity of 47.1%. sTIM-3 was found to be a sensitive marker for disease activity in SLE patients, as illustrated in **Table 4**

Table 1: Demographic and disease activity characteristics of the patients

Variables	**SLE** (No=112**)**
Age (years) Mean±SD	 33.43±8.85
Sex: No. (%) Female Male	 99 (88.4%) 13 (11.6%)
Duration (years) Mean±SD Median (IQR)	 5.05±4.29 3.5 (1-3.5)
SLEDAI-2K score Mean±SD Median (IQR)	 7.25±8.03 4 (0-4)
SLEDAI-2K grades: No. (%) No activity (0) Mild activity (1-5) Moderate activity (6-10) High activity (11-19) very High (≥20)	 31 (27.7%) 28 (25%) 22 (19.6%) 14 (12.5%) 17 (15.2%)
Treatment: No. (%) Corticosteroids Leflunomide Cyclophosphamide Azathioprine Hydroxychloroquine Mycophenolate mofetil Biological treatment	 98 (87.5%) 8 (7.1%) 14 (12.5%) 63 (56.3%) 104 (92.8%) 44 (39.3%) 3 (2.7%)

SLE: systemic lupus erythematosus, **SD:** standard deviation, **IQR:** interquartile range, **NO:** number, **SLEDAI-2K**: SLE disease activity index 2000

Table 2: Correlations between sTIM-3 levels and different SLE disease-related parameters

Characteristic	**STIM-3 level**	
	r	**p**
SLEDAI-2K score	0.290	0.041
ESR	0.277	0.084
24 hours urinary Protein	0.306	0.044
Anti dsDNA	0.240	0.172
C3	-0.004	0.982
C4	-0.149	0.357

SLEDAI-2K: SLE disease activity index-2000, **sTIM-3**: serum human T cell immunoglobulin mucin 3, **ESR:** erythrocyte sedimentation rate, Anti dsDNA: anti-double-stranded DNA, **C3:** compliment, **C4:** complement.

Table 3: Comparing sTIM-3 levels and SLEDAI-2K grading in SLE patients.

Characteristic	SLEDAI-2K Score grading					P value
	No activity	Mild	Moderate	High	Very high	
sTIM-3 concentration (pg\ml) Median (IQR)	477.25 (442.4-512.1)	443.78 (335-466.1)	779.5 (538.6-951.8)	513.5 (414.2-560.6)	518.4 (447.3-919.9)	**0.007***

SLEDAI-2K: SLE disease activity index-2000, **sTIM-3:** serum human T cell immunoglobulin mucin 3, **IQR:** interquartile range.

Table 4: Validity of sTIM-3 levels as a marker of disease activity in SLE patients.

Variables	AUC	95%CI	Cutoff	Sensitivity	Specificity	PPV	NPV	Accuracy
sTIM-3 concentration	0.662	0.494-0.831	458.43	73.9%	47.1%	65.4%	57.1%	62.5%

sTIM-3: serum T cell immunoglobulin mucin 3**, AUC:** area under the curve**, CI:** confidence interval, **PPV:** positive predictive value, **NPV:** negative predictive value.

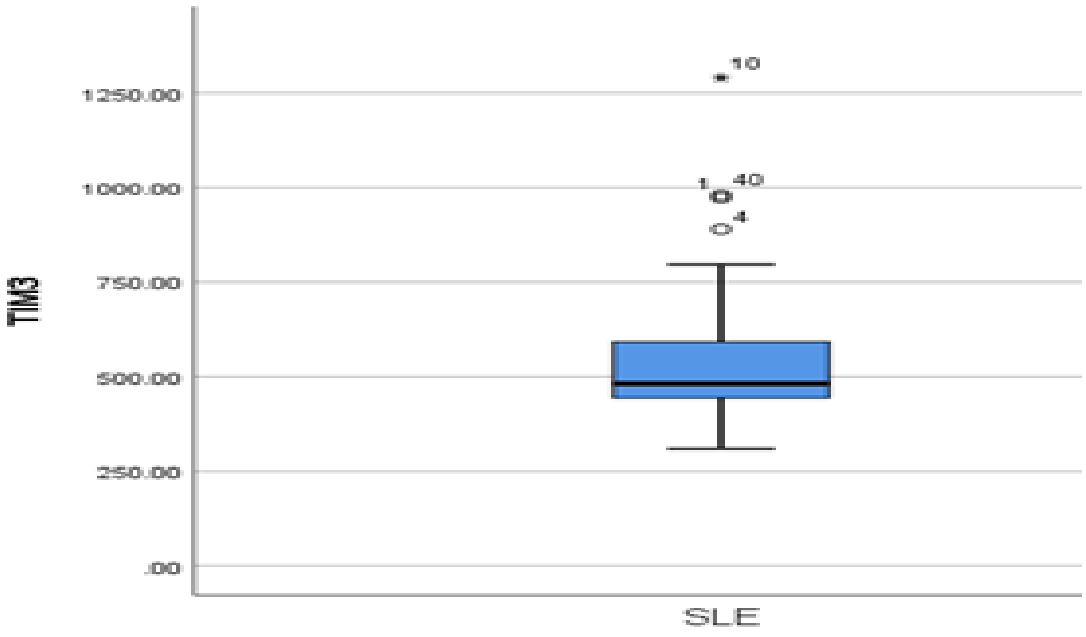

Figure 1: Box plot showing mean sTIM-3 levels in SLE patients.

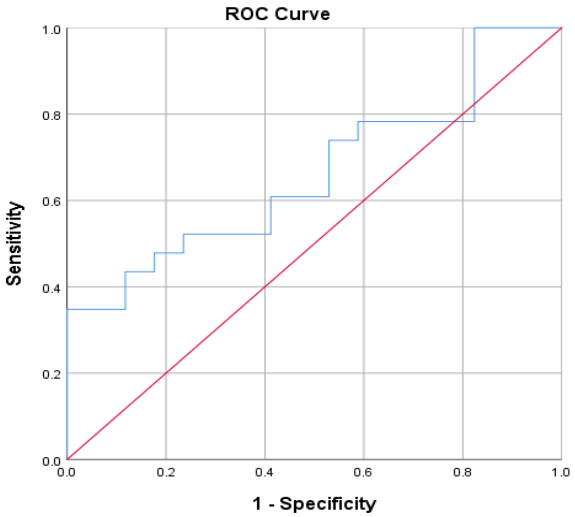

Figure 2: ROC curve illustrating the validity of sTIM-3 levels as a marker of disease activity for SLE patients.

DISCUSSION

SLE is a chronic autoimmune disease with immunological complexes in several organs, as multiple organs are typically involved in this condition, such as the musculoskeletal, circulatory, respiratory, neurological system, kidneys, and heart.[8] Though its exact cause is still unknown, SLE is believed to be caused by a mix of genetic, environmental, and hormonal variables. Predicting the occurrence of flares and remissions of SLE refers to a challenging task despite the cyclical pattern of the disease. Consequently, it is crucial to conduct a thorough study and develop a dependable biomarker for evaluating the extent of disease activity accurately.[9]

Through its negative control of the T cell response, TIM-3, which is found on the surface of terminally differentiated T cells, has been linked to the pathophysiology of Th1-driven disorders. The discovery that Gal-9 functions as a ligand for TIM-3 demonstrated the significance of the TIM-3/Gal-9 pathway as a regulator of Th1 response and immunological tolerance. SLE is typified as a Th1-dependent human autoimmune illness, which is marked by the deposition of immunological complexes and the generation of autoantibodies. While links between immunological co-signaling pathways and SLE have been found, the function of TIM-3 in the pathophysiology of SLE is yet unknown. 10 The aim of this research is to detect whether sTIM-3 level is elevated in individuals with SLE and whether this connection is related to disease activity.

In the current study, the median illness duration of SLE patients was 3.5 years, ranging from 1 to 3.5 years. In terms of clinical signs, the majority of cases (55%) reported arthritis. The majority of cases (92.8%) were treated with hydroxychloroquine, while corticosteroids were taken in 87.5% of cases. Among the SLE patients, the median SLEDAI-2K score was 4, and the mean score was 7.25 ± 8.03. Approximately 44.6% of cases exhibited mild to moderate disease activity, while 27.5% of cases were classified as high to very high activity.

This study illustrated that sTIM-3 levels were (550.96± 204.43 pg/mL) among SLE patients. The coinhibitory mechanism of TIM-3/Gal-9 is believed to have a significant role in regulating autoimmunity. The study of **Matsuoka et al.** revealed an increase in the T cell subset among persons diagnosed with SLE compared to the group of healthy people acting as controls. Additionally, there is a direct relationship between the presence of TIM-3 on T cells and the severity of SLE disease. The stimulation of the TIM-3/Gal-9 pathway functions as an anti-immune mediator in patients with SLE. [11.]

Asano et al. reported that individuals diagnosed with SLE have higher levels of TIM-3 in their bloodstream than healthy individuals. Furthermore, TIM-3 levels and the severity of SLE symptoms are clearly correlated. These results indicate a strong correlation exists between the beginning of active SLE and the blood level of TIM-3**.** [10]

In agreement with our results, **Asano et al.** revealed high levels of soluble TIM-3 in patients (2123 pg/mL). Ten, while disagreeing with our results, **Jin et al.** found a noteworthy decrease in serum sTIM-3 levels among lupus nephritis patients with SLE. Furthermore, no discernible association was seen between sTIM-3 levels and SLEDAI grades.[12]

This study demonstrated that the sensitivity of sTIM-3 concentration at a cutoff of 458.43, as a marker of SLE activity, was 73.9%. The level of specificity was 47.1%. There was a 65.4% positive predictive value (PVP) and 57.1% negative predictive value (PVN). The accuracy was 62.5%. It is a sensitive biomarker, but it is still not valid to discriminate against SLE patients with high disease activity. **Zhao et al.** used a ROC curve to study if sTIM-3 could distinguish between SLE patients at various stages of the illness. For sTIM-3, the AUC was 0.8021, with a cutoff value of 149.5 IU/mL, 75.27% sensitivity, and 81.82% specificity. [4]

According to our study, an evident and meaningful correlation was found between the sTIM-3 concentration and the SLEDAI-2K score, with statistical significance. In 24-hour urine samples, a statistically significant positive link was identified between sTIM-3 concentration and protein levels. In agreement with our results, **Asano et al.** demonstrated a strong association between blood sTIM-3 level and activity of disease as determined by SLEDAI-2K ($p < 0.001$, $r = 0.53$).[10]

CONCLUSION

Our research concluded that there was a significant association between sTIM-3 level and SLE disease activity. The results pointed to a strong correlation between the active SLE and the levels of circulating sTIM-3. However, it is still not valid to discriminate against SLE patients with high activity of the disease. This underscores the necessity for further investigation to ascertain the impact of this correlation on the intensity of SLE.

Declarations

Ethics approval: All procedures carried out in studies involving human subjects adhered to the Helsinki Declaration of 1964, its later amendments, and comparable ethical standards, as well as the ethical guidelines established by the institutional and/or national research committee.

Consent to participate: Every individual participant participating in the study gave informed consent.

Disclosure of potential conflicts of interest:

The authors confirm that they have no conflict of interest between them.

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Authors' contributions:

LK was responsible for the research conceptualization and proposal design; AZ and contributed to the data collection. NS and MM contributed to formal analysis and interpretation. LK, AZ and MR were responsible for writing and editing the original manuscript. MR was responsible for the final editing and revision. Lastly, all authors have collectively approved the final manuscript.

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