The Egyptian Journal of Hospital Medicine (April 2023) Vol. 91, Page 4601-4605

Assessment of Serum Syndecan-1 and its Relation to Renal Involvement in Patients with Systemic Lupus Erythematosus

Doaa Ebrahim Ahmed Shreif [1*], Nagat Mohamed ElGazzar [1], Rasha Adel El kholy [2], Doaa Shawky Alashkar [1]
[1] Rheumatology, Rehabilitation &Physical Medicine Department, Faculty of Medicine, Tanta University, Tanta, Egypt
2 Clinical Pathology Department, Faculty of Medicine, Tanta University, Tanta, Egypt
***Corresponding author: Doaa Ebrahim Ahmed Shreif**., **Mobile:** +201099823372, **E-Mail:** doaa.elghadour77@gmail.com

ABSTRACT
Background: Systemic lupus erythematosus (SLE) is an autoimmune illness with variable clinical symptoms and disease course. We aimed to identify the Syndecan-1(SDC-1) role in SLE patients with renal involvement, and to determine its correlation with the disease activity & severity. **Patients and Methods:** This case control research was performed on 30 SLE patients with renal involvement; they fulfilled the 2019 EULAR/ACR SLE Classification Criteria and were collected from outpatient and inpatient clinics of Department of Rheumatology, Rehabilitation, and Physical Medicine at Tanta University Hospitals. Twenty apparently healthy volunteers of matched age and sex were included as controls.**Results:** Serum level of SDC1 was significantly increased in SLE patients compared to controls. There was a significant negative correlation between SDC1 & C3 level, a significant positive correlation between SDC1 & WBCs, ESR, CRP, blood urea, serum creatinine, 24 h urinary protein, A/C ratio, Anti-dsDNA, SLEDI & illness duration.
Conclusions: The SDC1 serum level was significantly higher than controls, and positively correlated with disease activity In SLE patients with LN.
Keywords: Serum Syndecan-1, Renal Involvement, SLE.

INTRODUCTION
Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by variant clinical manifestations. Genetic, hormonal, environmental and viral factors are likely to play a role in SLE etiology; yet, the exact SLE pathoetiology remains unknown but it is believed to be multifactorial [(1)]. Lupus nephritis (LN) involves about 50% of SLE patients and is associated with substantial morbidity and mortality [(2)].

It has been shown that standard serological biomarkers, like anti-dsDNA antibodies & complement levels, are neither predictors of disease flares nor accurate indicators of disease activity [(3)].

Absence of effective SLE biomarkers impedes the disease activity and treatment response assessment. As a result, new biomarkers development to utilize as surrogate measures of disease activity and/or to predict illness flare-ups is of an increasing interest [(3)]. Early identification of renal impairment can enhance clinical outcomes & reduce the end-stage renal disease risk [(4)].

Therefore, for the effective management, an easily accessible, noninvasive & reproducible biomarker to anticipate the nephritis onset & measure its activity would be important [(5)]. Syndecan, a heparin -sulphate proteoglycan, participates in a variety of biological pathways, as inflammation, development and wound healing. [(6)]. Four SDC proteins are found in mammals encoded by 4 separate genes [(7)]. Of the 4 SDC subtypes; SDC-1 (CD138) is widely expressed on epithelial, endothelial, and plasma cell surfaces [(6)].In active SLE, serum SDC-1 was significantly higher than in inactive SLE, and its level was linked with SLE disease activity index & CD138-positive plasma cells ratio [(8)]. Under certain pathological situations, It is feasible to release the SDC-1 extracellular domain into extracellular fluids by removing it from the cell surface [(9)]. We aimed to identify the SDC-1 role in SLE patients with renal involvement, & to research its correlation with the disease severity & activity.

SUBJECTS AND METHODS
This case control research was performed on 30 SLE patients with renal involvement; they fulfilled the 2019 EULAR/ACR SLE Classification Criteria for SLE [(10)]. They were selected from the inpatient and outpatient clinics of Tanta University Hospitals' Department of Rheumatology, Rehabilitation, and Physical Medicine. Twenty sex and age- matched volunteers in an apparent good health were included as controls in this research.

Ethical considerations:

Informed written consent was obtained from each eligible patient who participated in the research. The current research was approved by Local Research Ethics Committee of Faculty of Medicine Tanta University at 2/10/2021 approved code 34951/10/21. All patients were given an explanation of each test, before obtaining their signed informed consent to participate in the research, Patients with stroke, transient ischemic attack, vasculitis, & pre-existing overt coronary artery disease (myocardial infarction, typical angina) and pregnant females were excluded from the research. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patients underwent the following assessments: **Full medical history taking:**

Name, age, gender, residence, occupation, and special habits), Complaint in the patient's own words, current history: onset, course, illness duration, family history of comparable condition or other rheumatologic disorders and past history of previous medications or operation, and **Complete clinical examination:**

Received: 19/11/2022
Accepted: 22/01/2023

General examination with stress on renal aspect and **musculoskeletal examination**.

Disease activity assessment:

Depending on the SLE Disease Activity Index (SLEDAI) [(11)].

Blood Sampling: Whole blood was collected by standard venipuncture in Blood Collection Tubes containing buffered sodium citrate solution for erythrocyte sedimentation rate (ESR) determination, K3EDTA for complete blood count (CBC) on Erma INC, PCE 210 N automated cell counter, and a tube containing clot activator/Sep, after centrifugation, one part of the serum samples was stored at −20 °C for assay of serum SDC-1 was estimated by ELISA kits from Shanghai Sunred Biological Technology Co., Ltd, Catalogue No. 201-12-5030, and the other part was immediately used for determination of blood urea and creatinine, urinary creatinine on fully automated chemistry analyzer, anti dsDNA on automated fluorescence immunoassay analyzers and C3,C4,CRP, and urinary protein on semiautomated nephelometry analyzer [(12)].

Method of assay of SDC1: Human SDC1 was estimated by ELISA kits from Shanghai Sunred Biological Technology Co., Ltd, Catalogue No. 201-12-5030, **Test principle:** By double ELISA kit, SDC1 in human samples was determined. Add SDC1 to the monoclonal antibody enzyme that has been precoated with the human SDC1 monoclonal antibody, and then incubate. Add biotin-labeled SDC1 antibody coupled with streptavidin-HRP to generate immunocomplex. Repeat incubation and washing to eliminate uncombined enzymes. Add chromogen solution A, B. There is a positive correlation between the color chroma & SDC1 concentration in the sample. The liquid's color changes from green to blue to yellow when exposed to acid.

Calculation of results: The mean absorbance was computed for each set of standards, controls, and samples using a Tecan Spectra II (Switzerland) Microplate Reader. After adding the stop solution, the blank optical density was subtracted under 450 nm wavelengths after 15 minutes. The percentage of absorbance was plotted on the y-axis & the standard concentration was displayed on the x-axis. The best-fitting straight line was drawn through the standard points.

Statistical analysis: SPSS v27 (IBM, Armonk, New York, United States) was used for the statistical analysis. To determine the data distribution, histograms & Shapiro-Wilks test was utilized. Qualitative variables were examined using the Chi-square test or Fisher's exact test, to examine quantitative parametric data reported as mean and SD, unpaired student t-test was used. To assess the non-parametric quantitative data reported as the median and IQR, Mann Whitney test was used as applicable. A two-tailed P value of less than 0.05 considered significant [(13)].

RESULTS

Age was insignificantly different between SLE patients & controls - Most of our patients were females (83.3%). Hb, platelets, ESR, CRP, serum creatinine, blood urea, C3, Anti-ds DNA, 24h protein in urine & A\C ratio were significantly different in the studied groups (P <0.05). **Table 1**

Table 1: Comparison of demographic and laboratory data in the studied groups

	SLE patients (n = 30)	Control (n = 20)	Test of Sig.	p
Sex Male Female	 5(16.7%) 25(83.3%)	 5 (25.0%) 15(75.0%)	χ^2= 0.521	> 0.05
Age	32.30 ± 9.0	38.75 ± 15.15	t= 1.713	> 0.05
Duration of illness	7.13 ± 3.15	---	---	----
Hemoglobin (g/dl), Ref range: (11-16)	11.09 ± 1.82	12.10 ± 1.0	t= 2.510*	< 0.05*
WBCs (×10³ /cmm), Ref range: (4 – 11)	6.34 ± 1.65	6.30 ± 1.52	t= 0.075	> 0.05
Platelets count (×10³), Ref range: (150 – 450)	194.0 (164.0 – 249.0)	221.5(204.0 – 290.0)	U= 201.0*	< 0.05*
ESR 1st hour (mm/h), Ref range: (1-15)	42.63 ± 4.75	9.25 ± 2.25	7.288*	<0.001*
CRP (mg/L), Ref range: up to 6	8.11 ± 1.91	5.15 ± 1.63	3.056*	< 0.05*
Blood urea (mg/dl), Ref range: (10 – 50)	31.0 (22.0 – 50.0)	17.0 (13.50 – 27.0)	U= 116.50*	<0.001*
Serum Creatinine (mg/dl),Ref range: (0. 6 – 1. 1)	1.09 ± 0.28	0.91 ± 0.16	t= 2.981*	< 0.05*
24 urinary protein (mg/24hrs urine) Ref range: (0 – 150)	1286.0 (600.0 – 1560.0)	86.0 (58.0 – 110.5)	U=13.00*	<0.001*
A/C ratio (mg/g. creat.) Ref range: less than 30	26.61(17.70–50.83)	15.25 (10.50 – 24.0)	U= 146.50*	< 0.05*
C3 (mg/dl), Ref range: (50 – 90)	102.9 ± 9.88	76.65 ± 4.92	t= 3.249*	< 0.05*
C4 (mg/dl), Ref range: (20 – 40)	25.80 ± 5.51	26.50 ± 5.12	t= 0.335	>0.05
Positive Anti dsDNA, Ref range: up to 30	80.0 (39.4 – 365.0)	12.0 (9.0 – 15.5)	t= 0.000*	<0.001*

Data are presented as mean ± SD or frequency (%) or median (IQR). WBCs: White blood cell, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, A/C: albumin creatinine, C: complement.*: statistically significant.

SDC1 was significantly different between SLE patients & controls (p < 0.05). **Table 2**

Table 2: Comparison between the two studied groups according to Syndecan-1 marker

	SLE patients (n = 30)	Control (n = 20)	U	P
SDC1 (ng/dl) **Ref range: up to 5.4**	7.55 (6.10 – 9.50)	5.55 (2.90 – 7.20)	151.00*	< 0.05*

SDC 1: Syndican-1 *: statistically significant.

SLE patients, there was a significant negative correlation between SDC1 & C3, a significant positive correlation between SDC1 and WBCs, ESR, CRP, serum creatinine, blood urea, 24h urinary protein, A/C ratio, Anti-ds DNA, SLEDI & duration of illness ($p < 0.05$), and insignificant correlation between SDC1 & age and sex, C4, Hb level and PLT count **Table 3 (a, b, c), Figures 1,2.**

Table (3a): Correlation between SDC1 with age, sex, haemoglobin, C4 and platelets in SLE patients' group (n = 30) (no correlation)

	r_s	P	
SDC1 vs. Age (years)	-0.186	>0.05	
Sex	**Median (Min. – Max.)**	**U**	**P**
Male	6.10 (3.60 – 10.80)	45.00	> 0.05
Female	7.70 (4.10 – 18.20)		
Hemoglobin (g/dl)	0.010	> 0.05	
C4 (mg/dl)	0.110	> 0.05	
Platelet count (×10³)	-0.004	> 0.05	

Table (3b): Correlation between SDC1 with C3 in SLE patients' group (n = 30) (negative correlation)

	r_s	P
C3 (mg/dl)	-0.431	< 0.05*

Table (3c): Correlation between SDC1 with duration of disease ,WBCs, ESR, CRP, Serum creatinine ,blood urea, 24 urinary proteins , A/C ratio, Anti-ds DNA and SLEDI in SLE patients' group (n = 30) (positive correlation)

	r_s	P
Duration of illness	0.522	< 0.05*
WBCs (×10³ /cmm)	0.429	< 0.05*
ESR (mm/h)	0.543	< 0.05*
CRP (mg/L)	0.392	< 0.05*
Serum Creatinine (mg/dl)	0.395	< 0.05*
Blood urea (mg/dl)	0.397	< 0.05*
24 urinary proteins (mg/24hrs urine)	0.420	< 0.05*
A/C ratio (mg/g. creat.)	0.401	< 0.05*
Anti- ds DNA	0.473	< 0.05*
SLEDAI	0.437	< 0.05*

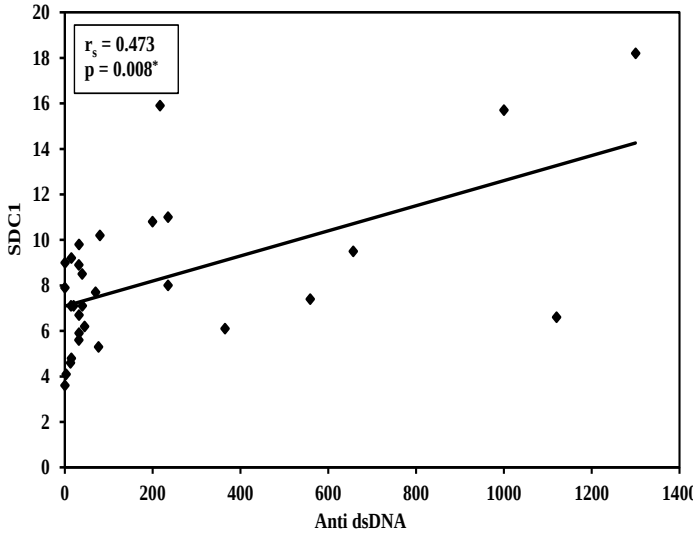

Figure 1: Correlation between SDC1 with Anti ds DNA in SLE patients

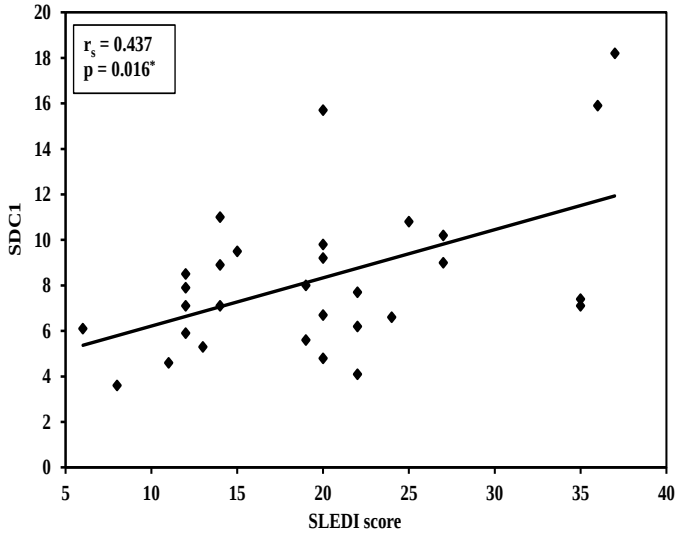

Figure 2: Correlation between SDC1 with SLEDI score in SLE patients group (n = 30)

DISCUSSION

LN is among the most severe organ-affecting manifestations of SLE. The majority of SLE patients acquire LN within 5 years of diagnosis, and LN is often treated with glucocorticoids and immunosuppressive medication. Despite growing understanding of illness causation and treatment, LN continues to be a major cause of morbidity & death among SLE cases [(14)].

SDC-1 (CD138), a heparan sulphate proteoglycan expressed on epithelial cells & endothelium surface. SDC-1 is higher in SLE cases, particularly when LN is released into the plasma due to glomerular endothelial glycocalyx breakdown caused by systemic inflammation. Following tissue damage, due to release and accumulation of several growth factors and proteases, SDC ectodomains are found in the inflammatory fluids [(15)].

Soluble SDC main function based mainly on the existence of Glycosaminoglycan chains which are transformed throughout ageing, cell development and illness. Moreover, the various sheddases are regulated in response to cell differentiation, inflammation and environmental stimuli [(16)].

MRL/Lpr mice between the ages of 4 and 6 weeks, develop lupus symptoms, as renal failure with positive anti-ds DNA antibodies, and the disease worsens with age due to fas apoptotic gene single mutation, develops autoreactive T- and B- cell activation and lymphoproliferation. Therefore, in MRL/Lpr mice of various ages, CD138 serum level analysis reveals that its concentration elevate with age. As shown in lupus patients, CD138 serum levels correlate closely with the onset of disease [(17)].

So, this research aimed to assess the SDC-1 serum level in SLE patients with LN and its association with disease activity' laboratory and clinical indicators.

Our research demonstrated that SDC-1 ranged from 1.0-12.6 ng/ml with median level of 5.55 and was significantly higher than controls **($p < 0.05$)**. which was supported by other studies such as **Salam *et al.* [(14)], Yu *et al.* [(18)]**

Epithelial, endothelial, and plasma cell surfaces express high levels of SDC-1 (CD138). In active SLE, serum SDC-1s were significantly raised, under certain pathological circumstances, like LN; the SDC-1 extracellular domain can be released into extracellular fluids [(19)].

These transmembrane proteins serve as coreceptors and adhesion molecules for several growth stimuli, hence enhancing the proliferation ability of the host cell. Transmembrane, cytoplasmic and extracellular domains are the constituents of protein. During normal cell turnover, the extracellular domain is frequently cleared from the cell surface. SDC-1 is a cytokine that has been linked to the development and survival of B lymphocytes. SDC-1 expression on B cells was associated with T follicular helper cells growth & illness progression [(16)].

In our research it was proven that there was no correlation between SDC1 with age and sex. Nevertheless, there was a positive correlation between SDC1 and illness duration. Contrary to the findings of **Yu *et al.* [(18)]**, SDC1 was not associated with patient age or SLE duration.

In addition, our research revealed a positive correlation between SDC1, acute phase reactant (ESR, CRP) and WBCs.

This was in agreement with **Yu *et al.* [(18)]** who found that SDC marker had positive correlation with disease activity, so it had a positive correlation with acute phase reactants.

We also found that SDC1 had a significant positive correlation with serum creatinine, blood urea, 24h urinary protein and A\C ratio.

Contrary to **Mosaad *et al.* [(20)]** who found insignificant correlation between the levels of SDC-1and renal function tests and proteinurea.

During normal cell turnover, the extracellular domain is continuously shed from the cell surface, resulting in the presence of Syndecan in circulation & indicating renal injury, so there was a positive correlation between Syndecan marker, renal function test & proteinuria [(15)].

However, its concentration was not considerably raised in SLE patients experiencing extrarenal flare showing that a high SDC-1 in SLE patients is independent to systemic inflammatory processes and is largely restricted to active nephritis [(15)].

Our results show that syndecan -1 marker was negatively correlated with C3 but not correlated with C4. This was in concordance with **Yu *et al.* [(18)]** who stated that Syndecan-1level showed an inverse correlation with C3 level.

In contrast to our research, no significant correlation between SDC-1 and C3 & C4 in the Egyptian juvenile SLE patients found by **Mosaad *et al.* [(20)]** .

The immune complexes formation results in complement activation in SLE, which lead to complement activation via the classical pathway, leading to its consumption during disease activity [(19)]. Syndecan -1 marker is positively correlated with disease activity so our marker was negatively correlated with complement level.

Regarding Anti dsDNA, we discovered a significant positive connection between SDC-1 & Anti dsDNA levels.

It is similar to **Yu *et al.* [(18)]** but in contrast with **Mosaad *et al.* [(20)]** who found no significant correlation between both markers.

SCD-1 may have a role in LN etiology as indicated in multiple studies. For example, a significant number of plasma cells that secrete anti-dsDNA antibodies were identified in the kidneys, and their numbers were associated with the serum dsDNA-IgG concentration [(15)].

A significant positive correlation between SDC1 & SLEDI score was indicated by our findings.

Our results came in line with **Mosaad *et al.*** [20] who found that there is significant positive correlation between SDC1 and SLEDI score.

The association of syndecan -1 with SLEDAI might be a consequence of the strong correlation between serum syndecan with anti-ds-DNA, proteinuria, renal function, acute phase reactant and complement level as mentioned, which are an important components of SLEDAI score.

CONCLUSION

SDC1 serum levels are significantly higher in SLE patients with LN than controls with positive correlation with illness duration & activity. To examine the SDC1 role in SLE pathogenesis, activity & LN, and its involvement in other inflammatory & autoimmune diseases, more research is required.

Statements:

- **All writers have given their permission to submit this work for publishing, and I certify that this is the case.**
- **Data and resources: Currently Existing**
- **Fund: There isn't any**
- **There are no conflicts of interest .**

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