

Semaphorin 5A, Anti-Nucleosome Antibodies and Ferritin: Disease Activity Markers in Patients with Systemic Lupus Erythematosus

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Abstract

Background: Systemic Lupus Erythematosus (SLE) is a multi-factorial, chronic autoimmune disorder characterized by dysfunction of T and B lymphocytes. Although the prognosis of SLE has improved in the past few decades, the absence of biomarkers for residual activity in various organs and the early detection of disease flares hamper further management. Conventional serologic markers of SLE, such as anti-dsDNA and complement levels, are not ideal, as they are not sufficiently sensitive and specific for the diagnosis and monitoring of disease activity. Thus, novel biomarkers for SLE activity have to be developed. Semaphorin 5A (Sema 5A), antinucleosome antibody (Anu A) and ferritin may fall into this category of novel bio markers.

Objective: To evaluate the role of serum Sema 5A, Anu A and ferritin in detection of SLE activity.

Methods: The present study included 40 patients with SLE divided according to Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) into two groups: 20 active SLE and 20 inactive SLE patients. They were compared with 20, sex and age matched healthy individuals for control. Levels of Sema 5A, Anu A were measured by Enzyme-Linked Immunosorbent Assay (ELISA) and serum ferritin levels were measured by Electrochemiluminescence immunoassay (ECLIA).

Results: There was highly significant increase in Sema 5A, Anu A and ferritin levels inactive when compared within active SLE patients and control subjects ($P=0.000$ and $P=0.000$ and $P=0.000$ respectively). There was a significant increase in ferritin level in inactive SLE patients when compared with control subjects ($P=0.045$) with no significant difference regarding Sema 5A, Anu A ($P=0.089$ and 0.225 respectively). There were positive correlations between Sema 5A, Anu A, ferritin and each of SLEDAI, and CRP. Positive correlation also found between Sema 5A and each of Anu A and ferritin and between AnuA and ferritin. Negative correlation was found between Sema 5A and C3 and between Anu A, ferritin and C4. Significant relation was found between Sema 5A, Anu A, ferritin and each of ANA and anti-dsDNA in active SLE patients. Area under the curve (AUC) for ferritin, Sema 5A and Anu A were (0.861, 1.0 and 1.0 respectively).

Conclusion: Our study showed that the serum proteins semaphorin 5A, antinucleosome antibodies and ferritin may be useful markers in monitoring disease activity in patients with SLE. Regarding area under the curve (AUC), these serum protein markers result in even better sensitivity and specificity profiles in picking up early relapse of SLE.

Keywords: Semaphorin 5A; Anti-nucleosome antibodies; Systemic lupus erythematosus

Introduction

Systemic Lupus Erythematosus (SLE) is a multisystem autoimmune disease that can potentially lead to serious organ complications and even death. Its manifestations are tremendously diverse, ranging from relatively mild cutaneous and articular involvement through to debilitating fatigue, cognitive impairment and end-stage renal disease. It is often called 'the disease of a thousand faces' [1].

Immunologically, SLE is characterized by loss of self-tolerance, high B cell reactivity with production of autoantibodies against nuclear and cytoplasmic components. Sustained production of autoantibodies leads to the accumulation of immune complex in many organs including kidney and brain [2]. Several types of cells and cytokines in the immune system have been confirmed to contribute to SLE pathogenesis [3].

Semaphorins comprise a large family of transmembrane and secreted proteins that have been described as axon guidance molecules during neuronal development and also involved in physiological processes such as organogenesis, immune cell regulation, and vascular

[4]. They are divided into eight classes based on sequence similarities and distinct structural features [5].

Semaphorin 5A (Sema 5A) belongs to class 5 that comprises transmembrane proteins which exhibit a unique extracellular domain containing 7 thrombospondin 1 repeats in addition to the sema domain. Sema 5A was described as promoting angiogenesis by increasing endothelial cell proliferation and decreasing apoptosis [6]. Previous studies revealed that Sema 5A act as immune semaphorin for its role in innate immune responses by inducing the expression of *TNF* and *IL-8* genes [7]. With respect to the significant role of secreted Sema 5A in innate immunity, increasing attention has been attracted to its role in immune regulation in autoimmune diseases. Recent studies showed that significantly elevated levels of secreted Sema 5A were detected in the serum of patients with Rheumatoid Arthritis (RA), active chronic Idiopathic Thrombocytopenic Purpura (ITP) and other autoimmune diseases and that soluble Sema 5A greatly promoted T cell and NK cell proliferation and induced the secretion of Th1/Th17 pro-inflammatory cytokines [3].

SLE is characterized by autoantibodies production, directed against nuclear antigens such as chromatin. The structural unit of chromatin is the nucleosome, which consists of a segment of dsDNA coiled around a histone core. Anti-chromatin autoantibodies are divided into two types: antibodies directed against the individual components of chromatin such as DNA and antibodies with a higher affinity to the intact nucleosome. It was demonstrated that the nucleosome is the primary antigen in SLE and that anti-dsDNA antibodies represent subset of this antibody population. These observations suggest that although anti-dsDNA antibodies are an accepted serological marker for the diagnosis and monitoring of SLE, anti-nucleosome antibodies may provide a better reflection of disease activity [8].

Ferritin is an acute-phase protein that is elevated in several disorders such as inflammation, autoimmune disorders, chronic infection and liver disease. It has an important role in host immune response. Higher ferritin levels have been observed in autoimmune diseases including RA, Multiple Sclerosis (MS), and Anti Phospholipid Syndrome (APS). The importance of ferritin in autoimmune diseases as a critical player in inflammation other than being an acute-phase reactant has been introduced recently [9].

Aim of the Study

To assess the role of serum Sema 5A, Anu A and ferritin in detection of SLE activity.

Subjects and Methods

This study was conducted on 40 patients with SLE who divided according to Systemic Lupus Disease Activity Index (SLDAI) into two groups: 20 active SLE patients and 20 inactive SLE patients selected from Rheumatology unit, Internal Medicine Department of Al-Zahraa and Al Demerdash Hospital during the period from Nov 2016 to Feb 2017.

Their age ranged from 38-66 y with mean \pm SD, 54.00 ± 8.96 y. They were compared with 20, sex and age matched healthy individuals as control group. An informed written consent was obtained from each patient and control subjects after explaining the purpose of the study which was approved by the local ethics committee.

Five mL of venous blood were withdrawn from each subject and was centrifuged and serum was obtained and stored at -20°C until assayed.

Sema 5A levels were measured by Enzyme Linked Immunosorbant Assay (ELISA) technique using kit supplied by Elab Science Biotechnology Co., Ltd. (catalog No: E1945Hu) according to manufacture instructions with sensitivity 0.02 ng/ml.

Anu A levels were measured by ELISA technique using kit supplied by SHANGHAI KORAIN Biotech Co., Ltd. (catalog No: E0504Hu) according to manufacture instructions with detection range 5 ng/ml-350 ng/ml and sensitivity 0.31 ng/ml.

ELISA assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Sema 5A or Anu A has been pre-coated onto a microplate. Standards and samples were pipetted into the wells and any Sema 5A or Anu A present was bound by the antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for Sema 5A or Anu A was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color develops in proportion to the amount of Sema 5A or Anu A bound in the initial step. The color development was stopped and the intensity of the color was measured. ELISA system used was (Reader A3 1851 and Washer 909) from Das (Italy).

Ferritin was measured on cobas e411 auto analyzer (electrochemiluminescence immunoassay "ECLIA") using kits supplied by Roche diagnostic (Roche diagnostic GmbH, D-68298, Mannheim, Germany).

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric and were presented as median with Interquartile Ranges (IQR) when their distribution found non-parametric. The comparison between two groups with qualitative data was done by using Chi-square test. The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test while data with non-parametric distribution compared between two groups using Mann-Whitney test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way Analysis of Variance (ANOVA) followed by *post hoc* analysis using LSD while groups with non-parametric data were compared by using Kruskal-Wallis test followed by *post hoc* analysis using Mann-Whitney test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. ROC curve was used to explore the sensitivity and specificity of tested markers.

Results

There was highly significant increase in Sema 5A and Anu A levels in active when compared with inactive SLE patients and control ($P=0.000$ and $P=0.000$ respectively) with no significant difference between inactive SLE patients and control ($P=0.225$ and 0.089 respectively).

There was highly significant increase in ferritin levels in active SLE patient when compared with inactive SLE patients and control subjects ($P1$ and $P2=0.000$ respectively) with significant increase in its levels in inactive SLE patients when compared with control ($P3=0.045$) (Table

1). There was highly significant difference between active and inactive SLE patients regarding SLEDAI and 24 h protein (P=0.000 and 0.000 respectively) (Table 2) (Figure 1).

		Active SLE group	Inactive SLE group	Control group	Chi-square test	P1	P2	P3	
CRP mg /l	Mean ± SD	23.71 ± 13.48	14.70 ± 6.30	6.00 ± 0.00	11.466*	0	0.004	0	0.03
	Range	12-48	6-24	6-6					
ESR mm/h	Mean ± SD	68.24 ± 15.95	32.05 ± 16.21	14.10 ± 4.99	84.179*	0	0	0	0
	Range	40-90	21-60	4-20					
ANA	Negative	8 (40.0%)	8 (40.0%)	17 (85.0%)	10.909	0.004	1	0.003	0.003
	Positive	12 (60.0%)	12 (60.0%)	3 (15.0%)					
Anti-dsDNA	Negative	2 (10.0%)	15 (75.0%)	20 (100.0%)	36.522	0	0	0	0.016
	Positive	18 (90.0%)	5 (25.0%)	0 (0.0%)					
C3 units/ml	Mean ± SD	62.71 ± 19.95	90.45 ± 19.38	139.85 ± 23.55	70.431	0	0	0	0
	Range	30-99	65-134	95-170					
C4 units/ml	Mean ± SD	32.95 ± 10.87	34.45 ± 7.86	26.45 ± 9.47	4.015	0.023	0.616	0.033	0.01
	Range	17-55	22-44	12-42					
Ferritin ng/ml	Mean ± SD	937.81 ± 627.60	286.05 ± 150.36	41.30 ± 20.47	30.871	0	0	0	0.045
	Range	199-2400	69-576	15-81					
Anu A-IgG ng/ml	Mean ± SD	293.95 ± 93.77	31.58 ± 14.45	1.08 ± 0.29	172.664	0	0	0	0.089
	Range	178-499	16.7-71	0.7-2					
Sema 5A ng/ml	Mean ± SD	8.34 ± 3.76	1.12 ± 0.23	0.26 ± 0.10	82.855	0	0	0	0.225
	Range	3.6-14.1	0.84-1.7	0.1-0.43					

*One Way ANOVA; P<0.05=significant; P1: Comparison between active SLE group vs. inactive SLE group; P2: Comparison between active SLE group vs. control group; P3: Comparison between inactive SLE group vs. control group.

Table 1: Comparison between active SLE patient, inactive SLE patients and control as regard laboratory data.

		Active SLE group	Inactive SLE group	Independent t-test	
		No=20	No=20	t	P-value
SLEDAI	Mean ± SD	11.29 ± 3.44	3.70 ± 1.34	9.216	0
	Range	7-18	1-6		
24 h proteins gm/24	Mean ± SD	0.82 ± 0.36	0.34 ± 0.11	5.672	0
	Range	0.3-1.8	0.2-0.54		

P<0.05=Significant.

Table 2: Comparison between active SLE patients and inactive SLE patients as regard SLEDAI, 24 h proteins.

In active SLE patients there was a positive correlation between ferritin and each of CRP, SLEDAI, Sema 5A and Anu A (P=0.000 for all) (Table 3) and significant relation with ANA and anti-dsDNA (P=0.009 and 0.044) (Table 4), but negatively correlated with C4 (P=0.024) (Table 3). A positive correlation also found between Anu A

and CRP and SLEDAI (P=0.000 and P=0.000) (Table 3) and significant relation with ANA and anti-ds DNA (P=0.003 and 0.04) (Table 4), but negatively correlated with C4 (P=0.035) (Table 3). In this group of patients we also found a positive correlation between Sema 5A and CRP and SLEDAI (P=0.000 and 0.000) (Table 3), and significant relation with ANA and anti-ds DNA (P=0.000 and 0.011) (Table 4), but negatively correlated with C3 (P=0.037) (Table 3). As regard in active SLE patients we found a positive correlation between ferritin and CRP and SLEDAI (P=0.005 and 0.002) (Table 5) and significant relation between Sema 5A and ANA (P=0.03) (Tables 5 and 6). Area under the curve (AUC) (which reflect the sensitivity and specificity of tested markers and interpreted as 0.90-1.0 is excellent biomarker, 0.80-0.90 is good biomarker) for ferritin, Sema 5A and Anu A were (0.861, 1.0 and 1.0 respectively) (Figures 2 and 3).

	Active SLE group					
	Ferritin ng/ml		Anu A-IgG ng/ml		Sema 5A ng/ml	
	r	P-value	r	P-value	r	P-value
HB gm/dl	-0.179	0.439	-0.282	0.216	-0.155	0.501

CRP mg/l	0.715**	0	0.915**	0	0.881**	0
ESR mm/h	0.03	0.897	0.143	0.538	0.152	0.511
C3 units/ml	-0.352	0.117	-0.603**	0.001	-0.458*	0.037
C4 units/ml	-0.489*	0.024	-0.462*	0.035	-0.304	0.18
SLEDAI	0.884**	0	0.967**	0	0.931**	0
24 h proteins gm/24	-0.093	0.687	0.035	0.882	-0.09	0.699

Ferritin ng/ml	-	-	0.816**	0	0.744**	0
Anu A-IgG ng/ml	0.816**	0	-	-	0.878**	0
Sema 5A ng/ml	0.744**	0	0.878**	0	-	-

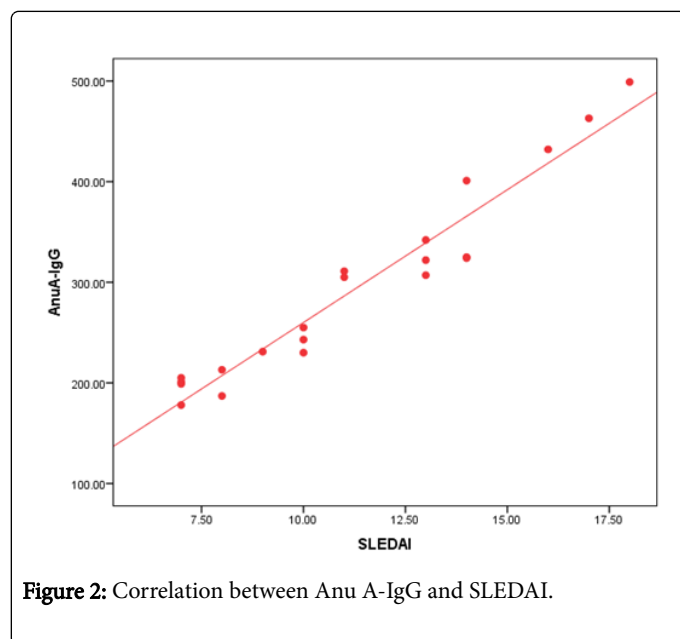
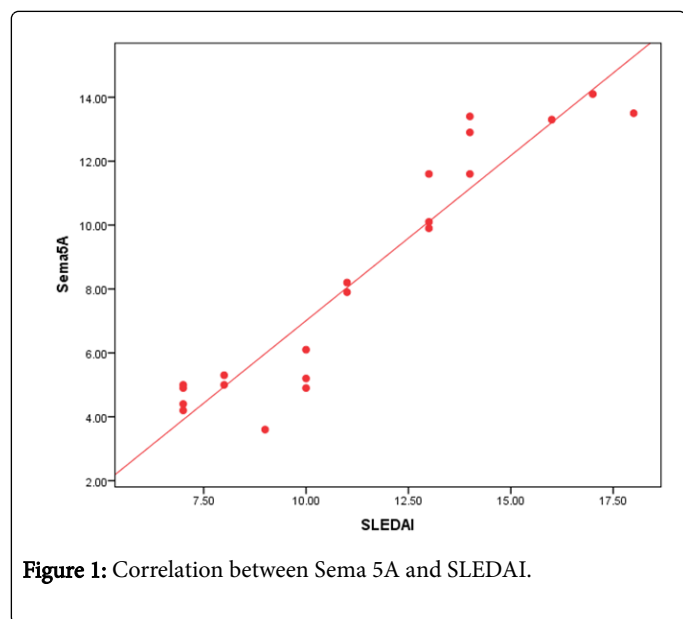
P<0.05=Significant.

Table 3: Correlation between Ferritin, Anu A-IgG, and Sema 5A and other studied parameters in active SLE patients.

Active SLE group		Ferritin ng/ml	P-value	Anu A-IgG ng/ml	P-value	Sema 5A ng/ml	P-value
		Mean ± SD		Range		Mean ± SD	
ANA	Negative	544.89 ± 328.73	0.009	228.33 ± 48.48	0.003	5.39 ± 1.59	0
	Positive	1232.50 ± 645.36		343.17 ± 90.07		10.55 ± 3.38	
Anti-dsDNA	Negative	273.00 ± 79.50	0.044	192.67 ± 12.74	0.04	4.53 ± 0.42	0.011
	Positive	1048.61 ± 609.63		310.83 ± 90.63		8.97 ± 2.69	

*One Way ANOVA.

Table 4: Relation between Ferritin, Anu A-IgG, Sema 5A and ANA, Anti-dsDNA in active SLE patients.



Inactive SLE group		Ferritin ng/ml	P-value	Anu A-IgG ng/ml	P-value	Sema 5A ng/ml	P-value
		Mean ± SD		Range		Mean ± SD	
ANA	Negative	300.45 ± 155.18	0.737	33.03 ± 17.04	0.725	0.99 ± 0.15	0.033
	Positive	276.44 ± 153.22		30.61 ± 13.18		1.21 ± 0.24	
Anti-dsDNA	Negative	292.21 ± 164.32	0.76	29.76 ± 12.65	0.344	1.09 ± 0.18	0.223
	Positive	267.56 ± 110.96		37.02 ± 19.57		1.23 ± 0.35	

*One Way ANOVA.

Table 5: Relation between Ferritin, Anu A-IgG, Sema 5A and ANA, anti-ds DNA in inactive SLE patients.

	Ferritin ng/ml		Anu A-IgG ng/ml		Sema 5A ng/ml	
	R	P-value	r	P-value	R	P-value
CRP mg /l	0.601**	0.005	0.176	0.458	0.25	0.289
ESR mm/h	0.294	0.208	0.214	0.364	0.258	0.272
C3 units/ml	0.237	0.315	-0.139	0.559	-0.336	0.147
C4 units/ml	0.368	0.11	-0.06	0.801	0.438	0.053
SLEDAI	0.646**	0.002	0.309	0.186	0.053	0.825
24 h proteins gm/24 h	0.232	0.325	-0.247	0.294	0.127	0.595
Ferritin ng/ml	-	-	0.4	0.073	0.241	0.293
Anu A-IgG ng/ml	0.4	0.073	-	-	-0.074	0.749
Sema 5A ng/ml	0.241	0.293	-0.074	0.749	-	-

Table 6: Correlation between Ferritin Anu A-IgG and Sema 5A and other studied parameters in inactive SLE patients.

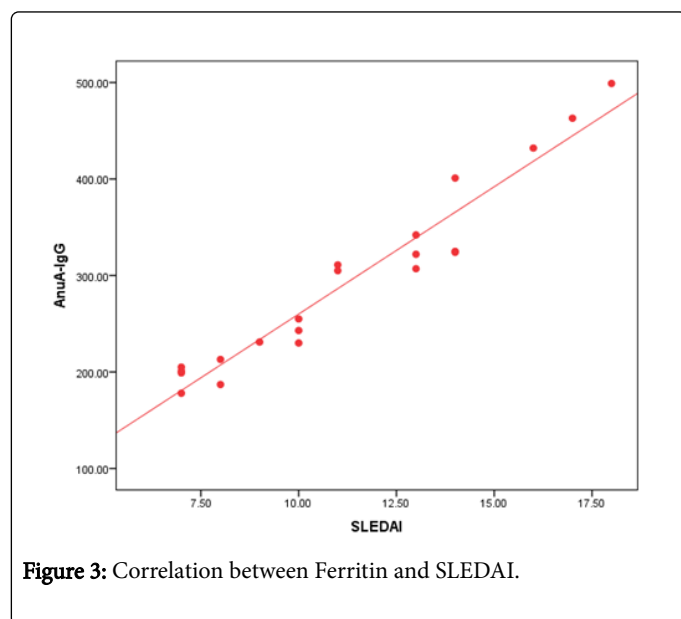


Figure 3: Correlation between Ferritin and SLEDAI.

Discussion

Semaphorins are a large family (classified into eight subclasses) of secreted and membrane bound proteins originally discovered in the nervous system, which are involved in repulsive axon guidance during nervous system development [3]. They were found in the micro environmental cells of the developing murine thymus, suggested that they are involved in the guidance of T precursor cells into the thymus by chemorepulsion [10].

Immune semaphorins are several members of the semaphorin family including Sema 3A, Sema 4D, Sema 6D, and Sema 7A essentially involved in immune cell regulation and might be involved in autoimmune disorders. Sema 5A has been shown to regulate several physiologic processes including angiogenesis and cancerogenesis but its role in the modulation of immune responses has remained unclear [6].

In this study, we demonstrated that serum levels of Sema 5A were significantly increased in active SLE patients when compared with inactive patients and controls (P=0.000). We also found that serum levels of Sema 5A were positively correlated with SLEDAI (r=0.931, P=0.000) and CRP (r=0.881, P=0.000), but negatively correlated with C3 (r=-0.458, P=0.037) in these patients. This is in accordance with Du et al. who reported the same results and concluded that elevated serum Sema 5A in SLE patients was correlated with disease activity and involved in the kidney and blood system damage. Thus the up-regulation of serum Sema 5A may serve as a biomarker of the disease activity and severity of SLE.

As regard Anu A, it has been demonstrated that they are increased in SLE patients, with positive correlation with global disease activity. In our study we found a significant increase in their levels in active SLE patients as compared with inactive SLE patients and control (P=0.000). This is in accordance with Li et al. who found that Anu A were significantly elevated in SLE patients *versus* controls and showed a moderate positive correlation with disease activity. He also concluded that these antibodies demonstrate greater fidelity as a biomarker for changes in SLE disease activity than traditional biomarkers, supporting the routine monitoring of this antibody in clinical practice. Our result also matched with Živković et al. who concluded that anti-nucleosome antibodies are associated with SLE and lupusnephritis activity, suggesting their potential usefulness in predictions of lupus nephritis and assessment of disease activity. We also found that Anu A positively correlated with SLEDAI (r=0.967 and P=0.000) and CRP (r=0.915 and P=0.000) and negatively correlated with C4 (r=-0.462 and P=0.035). A significant relation was found also between its level and ANA (r=3.453 and P=0.003) and anti-dsDNA (r=2.208 and P=0.04) and this matched with Li et al. who found a significant correlation between Anu A and SLEDAI, C3 and anti-dsDNA and he recorded that the changes in clinical state were not mirrored by changes in anti-dsDNA antibodies and in time-dependent analysis, anti-nucleosome antibodies showed a better fit over time than anti-dsDNA antibodies and complement.

In patients with active SLE, ferritin levels have been shown to be elevated and correlated with disease activity scores. However, the exact role of ferritin in the pathogenesis of SLE remains obscure [11].

The reason for elevated ferritin levels in SLE patients is poorly understood. Various pro-inflammatory cytokines like interleukin (IL)-6, IL-1a and interferon alpha (IFN-a) are shown to regulate expression and translation of ferritin [9]. The role of IL-6 and IFN-a in SLE is well documented as remaining elevated in SLE patients and positively correlated with disease activity [12-14]. Since SLE patients display higher levels of inflammatory molecules, it was hypothesized that enhanced ferritin serum levels in SLE patients could be correlated with inflammatory molecules like IL-6 and IFN-a [9]. In our study we

found a significant increase in ferritin levels in active SLE patients as compared with inactive SLE patients and control ($P=0.000$). This in accordance with Mok et al. who reported an elevated level of ferritin in active SLE patients when compared within active patients and healthy controls and demonstrated that ferritin had generally higher specificity and positive predictive value, but slightly lower sensitivity than conventional markers in detecting concurrent SLE activity. We found also that ferritin levels positively correlated with SLEDAI ($r=0.884$ and $P=0.000$) and CRP ($r=0.715$ and $P=0.000$) and negatively correlated with C4 ($r=-0.489$ and $P=0.024$) and C3 ($r= P=$) in active SLE patients. We found also a significant relation with ANA ($r=2.913$ and $P=0.009$) and anti-ds DNA ($r= 2.155$ and $P= 0.044$).

These results were matched with Tripathy et al. who found a significant correlation between ferritin and SLEDAI, C4, C3 and anti-dsDNA in SLE patients and concluded that serum ferritin is an excellent marker of disease activity and renal dysfunction in SLE and with Mok et al. who found significant correlation between ferritin and SLEDAI, anti-dsDNA and C3.

Conclusion

Our data indicates that serum proteins Sema 5A, Anu A and ferritin may be useful in the monitoring of disease activity in patients with SLE. Regarding area under the curve, these serum protein markers result in even better sensitivity and specificity profiles in picking up early relapse of SLE.

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