Biomarkers assay for identification and prediction of flare in patients with Systemic lupus Erythematosus

Nashwa Noreldin[1] Samah elshweek[1] and Mohamed M. Attia [2]

[1]Department of Internal Medicine, College of Medicine, University of Tanta, Tanta, Egypt
[2] Department of clinical pathology, College of Medicine, University of Tanta, Tanta, Egypt
nashwanor@hotmail.com

Abstract: Background/Aim: There is no reliable laboratory test to predict the beginning or the end of Systemic Lupus Erythematosus (SLE). Soluble vascular cell adhesion molecule 1 (sVCAM1) is a new promising marker of SLE activity The aim of this work is to study sVCAM1 in SLE patients and to copmare with other traditional markers. **Methods:** Thirty SLE patients divided according SLE Disease Activity Index (SLEDAI) into 20 SLE patients with active disease had SLEDAI above 5 (group I) and 10 SLE patients with inactive disease had SLEDAI less than 5 (group II) were compared versus 10 controls (group III) regarding Anti ds-DNA antibodies, Complement 3 (C3) & Complement 4 (C4) and soluble vascular cell adhesion molecule 1 (sVCAM1). All markers and disease activity index SLEDAI score, C3, C4, sVCAM1 & Anti-ds DNA levels were measured 3 times over 6 months ,at the beginning (the baseline measurement) and 3 and 6 months after joining the study. **Results:** Anti ds DNA and sVCAM1 were significantly increased while serum levels of C3 & C4 were significantly decreased in group I compared to group II and III .There was significant positive correlation between SLEDAI score and Anti ds DNA and sVCAM1 in groups I and II. Also there was significant negative correlation between SLEDAI score and serum levels of C3 & C4 in group I but not in group II. At the end of the follow up visits, patients were classified into two subgroups according to SLEDAI score: score less than or equal 5 (inactive group) and score more than 5 (active group). At the end of the follow up visits, patients were classified into two subgroups according to SLEDAI score: score less than or equal 5 (9 patients) and score more than 5 (7 patients). The statistical analysis between the two groups showed that the serum levels of Anti ds DNA was insignificantly lower in the inactive group while C4 levels were insignificantly higher. On the other hand, sVCAM1 was significantly lower in the inactive group while C3 levels were significantly higher. **Conclusion:** sVCAM1 is important laboratory parameters for assessing disease activity especially in patients with anti-dsDNA negative. Serial estimation of these serological markers helps in predicting lupus flare during follow up.

[Nashwa Noreldin, Samah elshweek and Mohamed M. Attia. **Biomarkers assay for identification and prediction of flare in patients with Systemic lupus Erythematosus.** *J Am Sci* 2014;10(10):105-111]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 18

Key words: systemic lupus erythematosis, sVCAM1, Anti ds DNA, C3, C4.

1. Introduction

Systemic Lupus Erythematosus (SLE) is a chronic, usually life-long, autoimmune disease characterized by unpredictable exacerbations and remissions with variable clinical manifestations. In SLE, there is a high probability for clinical involvement of the joints, skin, kidney, brain, lung, heart, serosa and gastrointestinal tract. (1) The SLE Disease Activity Index (SLEDAI) is an index that measures disease activity by weighting the importance of each organ system involved. This tool has been shown to be reliable and reproducible when used by various investigators and sensitive to change in a patient's condition. However SLEDSAI has certain limitations in that it does not score some life threatening manifestations such as pulmonary haemorrhage and haemolytic anaemia. Also, SLEDAI can be problematic at times in that the score may be the same whether the patients are improving, stable or worsening.(2,3) In addition, SLEDAI does not account for subjective symptoms such as fatigue, arthralgia, or myalgia, which might genuinely reflect lupus activity, and may be of high importance to patients. (4)

In SLE, the body's immune system produces antibodies against itself, particularly against protein in the cell nucleus .SLE is triggered by environmental factors that are unknown. These stimuli begin a reaction that leads to destruction of other cells in the body and exposure of their DNA, histones, and other proteins (particularly parts of the cell nucleus). The body's sensitized B-lymphocyte cells will now produce antibodies against these nuclear-related proteins. Antinuclear antibodies (ANA) are most characteristic and present in more than 95% of patients. Anti-double stranded DNA (ds-DNA) and Anti smith antibodies (anti-Sm antibodies) are unique to patients with SLE. (5) Also, there are other new markers of SLE disease activity, one of them is soluble vascular cell adhesion molecule 1 (sVCAM1) which is a member of immunoglobulin family and plays a role in cell to cell and in cell to extracellular matrix mediated immune responses. (6) In spite of that, there is no specific

laboratory test with reliable capability to identify or predict the beginning or the end of disease flare and hence a significant for doctors is there so as to find out tools for measuring activity and flare in this problematic disease.

The aim of this work is to study the level of Complement 3&4 (C3&C4), Anti-dsDNA antibodies as traditional markers together with the new marker sVCAM1 in SLE patients during activity and remission and to correlate those biomarkers with SLEDAI score in attempt to detect the beginning or the end of disease flare

2. Methods

After approval of the local ethical committee and obtaining a written informed consent, we investigated 30 patients admitted to the internal medicine department or presented to its outpatient clinic and 10 healthy individuals from January 2013 to December 2013. The diagnosis of SLE was established according to the revised American Rheumatism Association criteria while disease activity. Patients with SLE Disease Activity Index (SLEDAI) (reference 105) less than 5 were considered without activity. Exclusion criteria included age under 15 years, pregnancy, other autoimmune diseases, concomitant viral or bacterial infection at the time of study .Our study population was divided into three groups: group I: 20 SLE patients with active disease, group II: 10 SLE patients with inactive disease and group III: 10 healthy individuals as a control group.

All subjects were subjected to full history taking and clinical examination and laboratory investigations that included complete blood picture, liver function tests, renal function tests, erythrocyte sedimentation rate, C-reactive protein, urine analysis & 24 h protein in urine, electrocardiogram and chest X ray. Measurements of specific biomarkers were done for all subjects that included, Anti ds-DNA antibodies, Complement 3 (C3) & Complement 4 (C4) and soluble vascular cell adhesion molecule 1 (sVCAM1).

Anti ds-DNA antibody was measured by enzyme-linked immunosorbent assays kit supplied by Calbiotech , (catalog NO. DD037G), according to manufacturer instructions. Values up to 40 u/ml were considered negative. C3 & C4 were measured using turbidimetry technique; their kits were supplied by BioSystems; catalog .No COD 31084 and 31085 respectively. Normally C3 values range from 90 to 180 mg/dl while C4 values range from 10 to 40 mg/dl. Soluble VCAM-1 was measured using enzyme-linked immunosorbent assays (R&D Systems, Abingdon, UK) according to the manufacturer's protocol. Interassay and coefficients of variation were 8% and 6%, respectively.

Disease activity in SLE patients was scored using the SLEDAI to assess lupus activity. SLEDAI is a global score reflecting all aspects of disease activity. It is a weighted scale for 24 parameters and the score can range from zero to 105. Various manifestations are scored based on their presence or absence in the previous ten days of evaluation. Higher scores indicate more severe disease activity. The following definitions of outcomes were based on changes in the SLEDAI index: improvement is a reduction in SLEDAI of > 3, persistently active disease is a change in SLEDAI 3 and Remission is a SLEDAI of 0. Activity categories have been defined on the basis of SLEDAI scores: No activity (SLEDAI = 0), Mild activity (SLEDAI = 1-5), Moderate activity (SLEDAI = 6-10), High activity (SLEDAI = 11-19) and Very high activity (SLEDAI 20).

All marker and disease activity index SLEDAI score, C3, C4, sVCAM1 & Anti-ds DNA levels were measured 3 times over 6 months ,at the beginning (the baseline measurement) and 3 and 6 months after joining the study.

Statistical analysis of the data of the present study was conducted with SPSS V.16. using the mean, standard deviation and chi-square test . The relationship between inflammatory markers levels and SLEDAI was determined using the Spearman correlation analysis and the linear regression method .P values <0.05% were considered to be statistically significant.

3. Results

Forty eligible patients were included in this study. Age and gender were comparable in both groups whereas duration of the disease was significantly higher and SLEDAI score was significantly lower in group II compared to group I (Table 1).

Anti ds DNA and sVCAM1 were significantly increased while serum levels of C3 & C4 were significantly decreased in group I compared to group II and III (Table 2). There was significant positive correlation between SLEDAI score and Anti ds DNA and sVCAM1 in groups I and II. Also there was significant negative correlation between SLEDAI score and serum levels of C3 & C4 in group I but not in group II. (table 3) (Fig 1).

During follow up visits, two patients were missed while two other patients were died. At the end of the follow up visits, patients were classified into two subgroups according to SLEDAI score: score less than or equal 5 (9 patients) and score more than 5 (7 patients) (6 patients had manifested renal affection and 1 patient had psychosis) .The statistical analysis between the two groups showed that the serum levels of Anti ds DNA was insignificantly lower in the inactive group while C4 levels were insignificantly higher. On the other hand, sVCAM1 was significantly

lower in the inactive group while C3 levels were significantly higher. (Table 4) (Figures 2-5)

Table 1: Demographic and basal characteristics

Variable	Group I (N:20)	Group II (N:10)	Group III (N:10)	*P*-value
Age (years) (Range, mean ± SD)	17.00 - 45.00 27.45 ± 7.80	20.00 - 38.00 29.70 ± 6.45	18.00 - 35.00 25.80 ± 5.12	0.454
Gender M/F Number,/%	1 (5%)/19(95%)	2(20%)/8(80%)	3(30%)/7(70%)	0.161
Duration OF SLE in months (Range, mean ± SD)	1.00- 48.00 21.300± 6.416	8.00 -72.00 37.40±19.82	-	<0.001*
SLEDAI Score	10.00- 31.00 21.30± 6.42	0.00-6.00 2.60±2.84	-	<0.001*

*= significant

Table 2: Comparison between group I, group II & controls as regard serum level of C3,C4 , Anti ds DNA& sVCAM1.

	Group I (20)	Group I (20)	Group III (10)	*ANOVA*	
				F	*P*-value
Anti ds DNA	298.3±198.87	57.09±35.49	23.770±8.094	15.053	<0.001*
C4	8.44±3.16	24.31±7.48	24.810±8.364	27.317	<0.001*
C3	47.75±21.05	115.52±16.50	125.330±28.418	58.424	<0.001*
sVCAM1	1317.75±224.77	861.7±210.87	502.1±121.609	58.09	<0.001*

Data are mean ± SD, *=significant

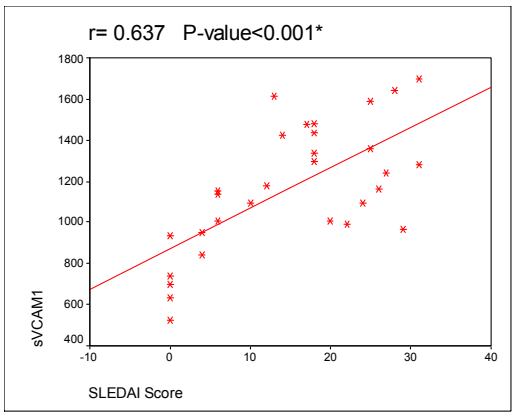
Table 3: Correlation between SLEDAI score and Anti ds DNA, C3, C4 and sVCAM1 in both groups

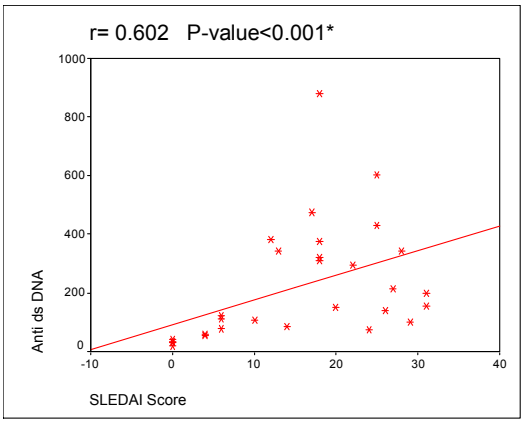
	Group I		*Group II*	
	r	*P* -value	*r*	*P* -value
Anti ds DNA	0.602	0.000*	0.909	0.000*
C4	-0.725	0.000*	0.331	0.351
C3	-0.753	0.000*	0.300	0.399
sVCAM1	0.637	0.000*	0.838	0.002*

Table 4: Comparison between patients in remission with those who still in active disease

marker		active SLE ,(n= 7) SLESLEAI more than 5 Mean (SD)	SLE remission ,(n=9) SLESLEAI less than 5 Mean (SD)	2-way RANOVA		
				Source	F	P-value
Anti ds DNA	Baseline	368.871 (160.402)	298.666 (243.186)	GROUPS	0.96946	0.370031
	3months	114.742 (47.751)	142.666 (88.901)	TIMES	12.21414	0.01738*
	6months	69.714 (47.804)	59.444 14.638	GROUPS TIMES	0.691961	0.443399
C3	Baseline	65.85 (15.258)	48.222 (23.599)	GROUPS	1.536298	0.270172
	3months	74 (20.008)	62.811 (19.696)	TIMES	21.90244	0.005434*
	6months	68.571 (24.589)	92.844 (8.203)	GROUPS TIMES	13.26003	0.014881*
C4	Baseline	8.485 (3.808)	8.933 (4.404)	GROUPS	1.871624	0.243104
	3months	14.1 (4.175)	14.522 (4.231)	TIMES	19.91615	0.011137*
	6months	13.085 (4.287)	24.311 (7.015)	GROUPS TIMES	0.544299	0.501596
sVCAM1.	Baseline	1510.714 (105.253)	1235.555 (229.357)	GROUPS	62.03203	0.00053*
	3months	1478.142 (67.422)	973.444 (196.518)	TIMES	49.17964	0.000909*
	6months	1399.571 (80.570)	682.222 (155.488)	GROUPS TIMES	20.24929	0.0064*

*=significant





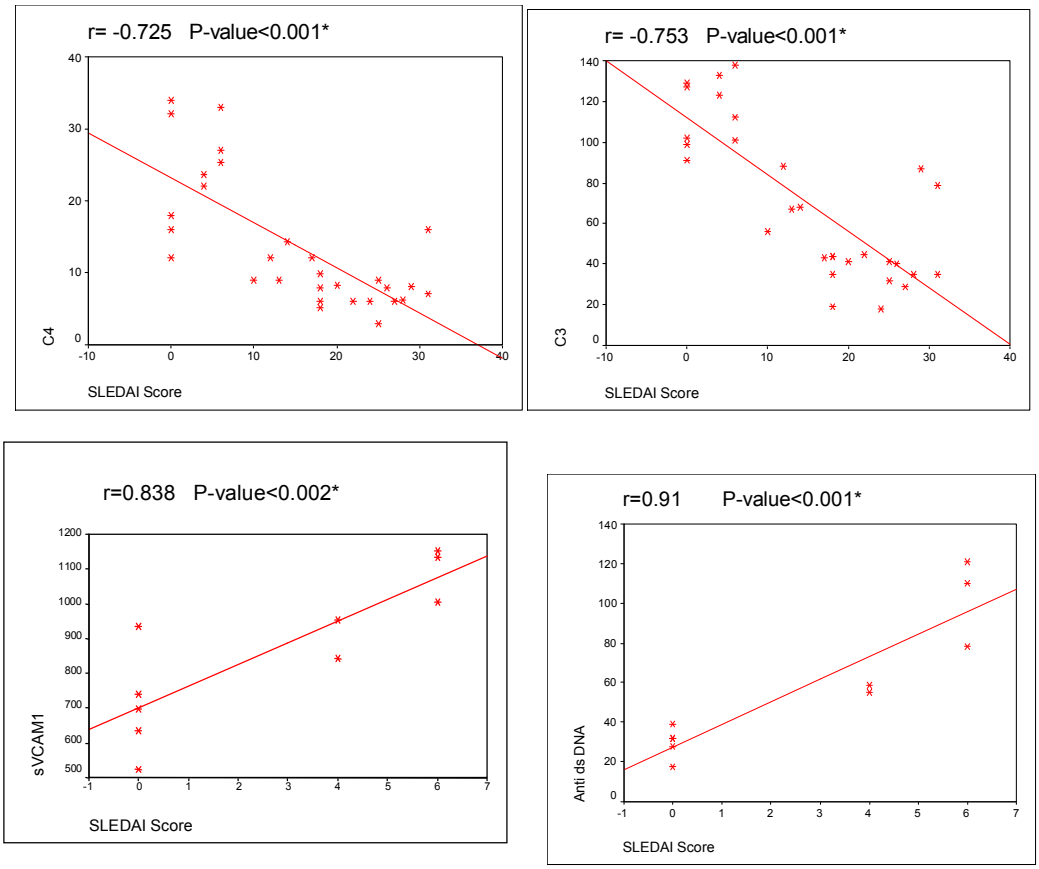


Figure 1: Correlations between SLEDAI and sVCAM1, Anti ds DNA, C4, C3 in group I and between SLEDAI and sVCAM1, Anti ds DNA in group II respectively.

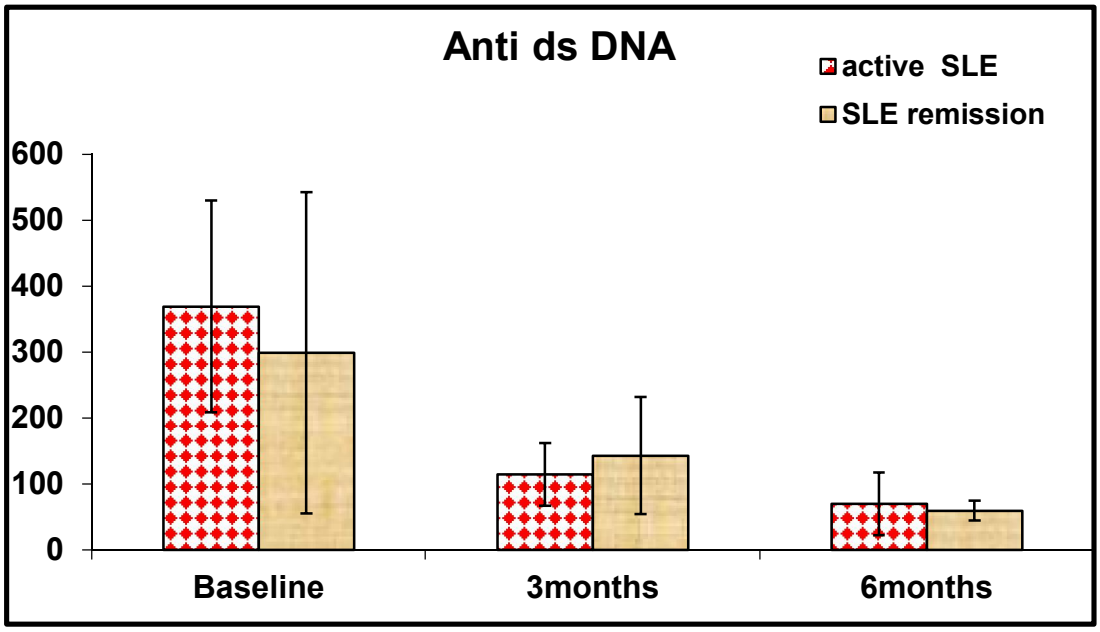


Figure 2: Comparison Anti ds DNA level, between patients in remission with those who still in active disease

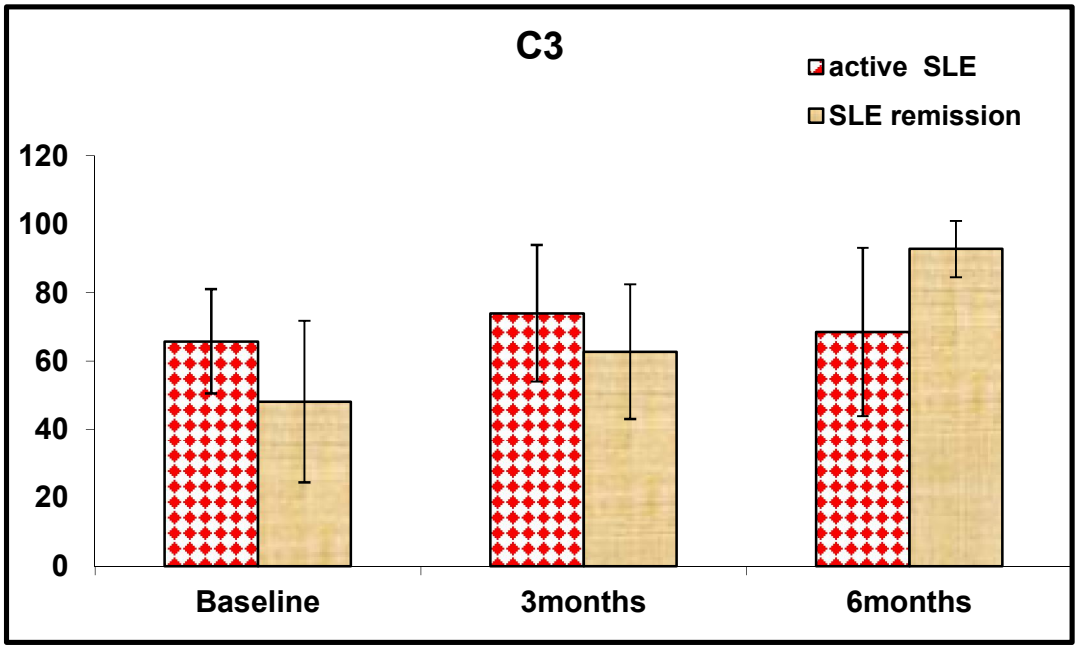


Figure 3: Comparison C3 level, between patients in remission with those who still in active disease

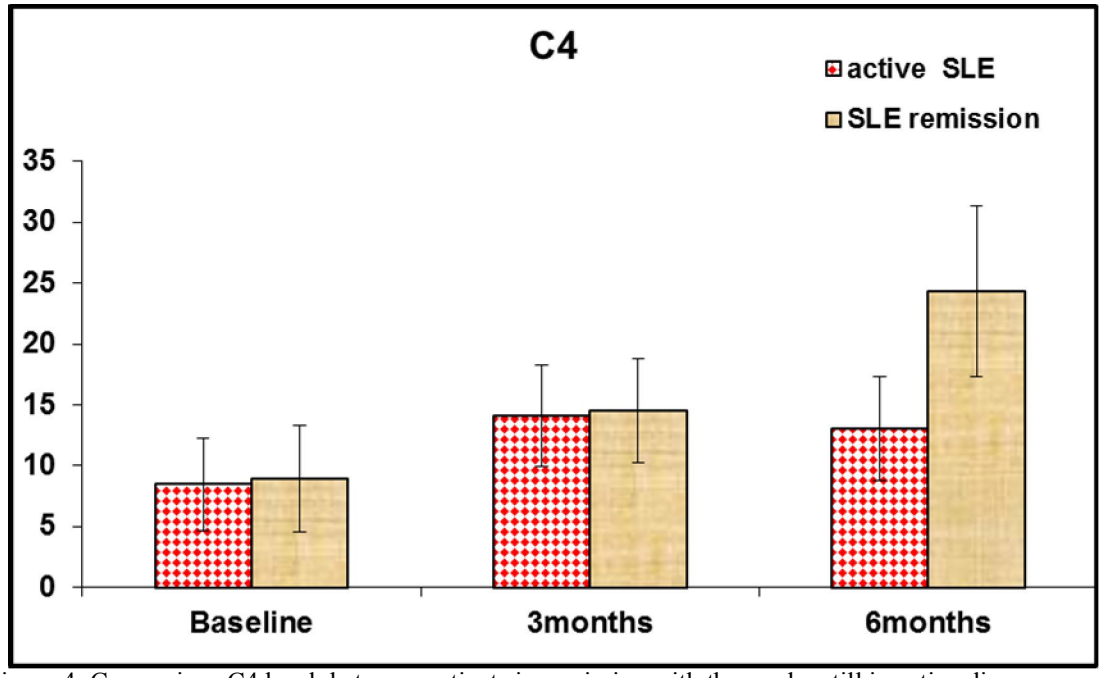


Figure 4: Comparison C4 level, between patients in remission with those who still in active disease

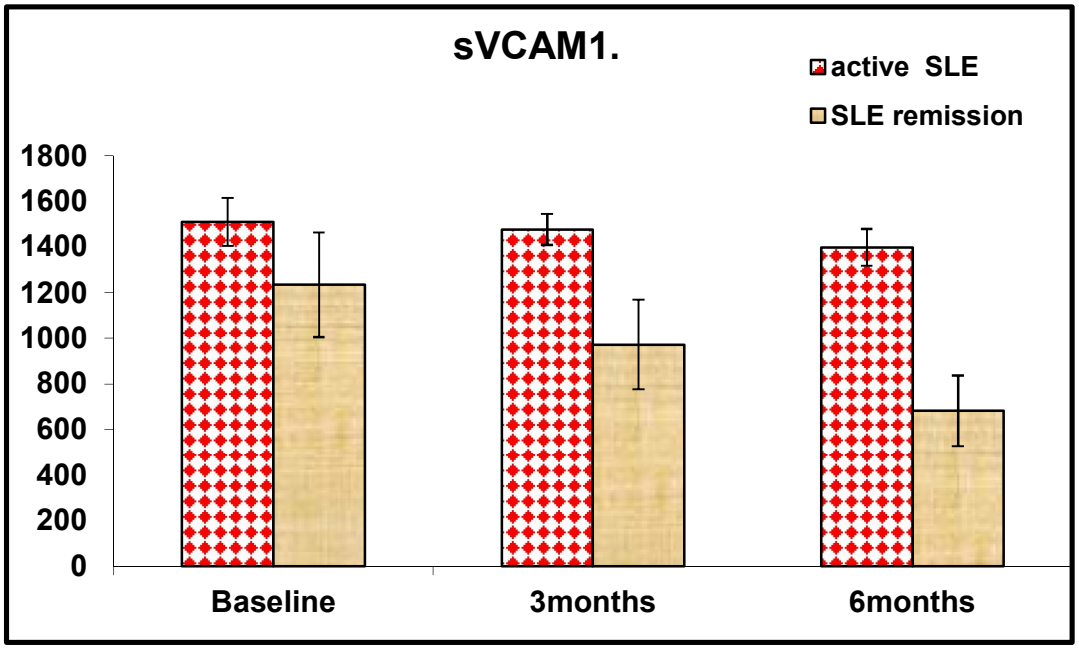


Figure 5: comparison of sVCAM1 level, between patients in remission with those who still in active disease

4. Discussion

Systemic lupus erythematosus (SLE) is characterized by the production of a wide range of autoantibodies. More than 100 different autoantibodies have been identified in the sera of patients with SLE. It is characterized by immune dysregulation resulting in the production of antinuclear antibodies, generation of circulating immune complexes ,auto-antibodies(Anti DNA), and activation of the complement system.(7,8) In our study, active SLE was associated with significantly increased levels of Anti-dsDNA antibodies with significant positive correlation between SLEDAI scores and anti-ds DNA levels. These findings are in agreement with previous studies. (9-11) on other hand, others(12-16) reported that anti ds DNA levels failed as reliable markers in assessing disease activity . In fact, measurement of anti-dsDNA antibodies is a sensitive method in the longitudinal follow-up of SLE patients, however, it is remarkable that a substantial proportion of SLE patients are anti-dsDNA negative.

Our results showed that serum levels of C3& C4 were significantly low in patients with activity as compared with those without activity and healthy one with significant negative correlation between C3 and C4 levels in lupus flares. Others.(17,18) concluded in their studies that changes in anti-dsDNA and complement concentrations were reported predominately to accompany flare up of lupus nephritis and a significant correlations have been found between these biological laboratory markers and some BILAG organ system scores& SLEDAI scores.(15,17) Adhesion molecules, including vascular cell adhesion molecule 1 sVCAM-1, E selectin and intercellular adhesion molecule 1 sICAM-1 are essential for cellular interactions and play an important role in the activation and adhesion of cells. Overproduction of sVCAM-1 could contribute to endothelial injury. Moreover, sVCAM-1 was proposed as a new marker of disease activity or therapeutic response in SLE(19)

Our results revealed that serum concentration of sVCAM-1 was significantly higher in active than inactive SLE patients or healthy control and, sVCAM-1 had correlation with SLEDAI. High level sVCAM-1 in active SLE patients appears to be a good marker of disease activity. In addition, during follow up of patients sVCAM-1 was still high in patients with SLE score above 5 and it, likewise, appears to correlate with the renal and vascular activity and probably reflects endothelial cells activation and increased their expression of adhesion molecules. Our results are in agreement with Elwy et al(6) where , in their a cohort study of 43 SLE patients (19 with inactive and 24 with active SLE) and 20 healthy controls, they concluded that serial measures of soluble vascular cell adhesion molecule (sVCAM-1) were significantly associated with SLE disease activity, and that both anti-dsDNA and sVCAM-1 were relatively good markers of disease activity in SLE and could help to predict remission or to monitor the therapeutic response. Furthermore Zaccagni et al(20) found that persistently elevated levels of sVCAM-1 over a 2 years period were associated with higher SLE related damage scores

compared to the group without persistently higher levels.

In conclusion: sVCAM-1 is an important laboratory parameter for assessing disease activity especially in patients with anti-dsDNA negative.

Acknowledgement:
We thank all staff members in Tanta University Hospital for their help in this work

Source of Support: Nil.
Conflict of Interest: We declare no conflict of interest.

References

1. Von Feldt JM. "Systemic lupus erythematosus: recognizing its various presentations,". Postgrad Med 1995;97:79-94 .
2. Ward MM, Marx AS, Barry NN. Comparison of the validity and sensitivity to change of 5 activity indices in systemic lupus erythematosus. J Rheumatol 2000;27:664-70.
3. Fortin PR, Abrahamowicz M, Clarke AE, et al. Do lupus disease activity measures detect clinically important change? J Rheumatol 2000;27:1421-8.
4. Chang E, Abrahamowicz M, Ferland D, et al. CaNIOS Investigators. Comparison of the responsiveness of lupus disease activity measures to changes in systemic lupus erythematosus activity relevant to patients and physicians. J Clin Epidemiol 2002;55:488-97.
5. Anisur Rahman, Ph.D., David A, et al. Mechanisms of Disease Systemic Lupus Erythematosus. N Engl J Med 2008; 358:929-39.
6. Elwy MA1, Galal ZA, Hasan HE. Immunoinflammatory markers and disease activity in systemic lupus erythematosus: something old, something new. East Mediterr Health J. 2010;16:893-90 .
7. Tan EM. Autoantibodies to nuclear antigens (ANA): their biology and medicine. Adv Immunol 1982;33:167.
8. Hahn BH. Antibodies to DNA. New Engl JMed 1998;338:1359–68.
9. Col K Narayanan, Col V Marwaha, Col K Shanmuganandan, et al. Correlation between Systemic Lupus Erythematosus Disease Activity Index, C3, C4 and Anti-dsDNA Antibodies. MJAFI 2010; 66: 102-107.
10. Siavash Nasiri ,Mansoor Karimifar ,Zahra Sayed Bonakdar. et al. Correlation of ESR, C3, C4, anti-DNA and lupus activity based on British Isles Lupus Assessment Group Index in patients of rheumatology clinic Rheumatol Int 2010 30:1605–1609.
11. Richard Hughes, Sarea UI-Hassan. Anti-dsDNA antibodies: their role in the detection and monitoring of SLE. Autoimmnity 2006;18:12.
12. Liu CC, Manzi S, Ahearn JM. Biomarkers for systemic lupus erythematosus: a review and perspective. Curr Opin Rheumatol. 2005;17(5):543–549.
13. Illei GG, Tackey E, Lapteva L, et al . Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. Arthritis Rheum. 2004;50:2048–65.
14. Magder LS, Ho A, Barr SG, et al. Decreases in anti–double stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. Arthritis Rheum. 2001,44:2342-9.
15. Esdaile JM, Joseph L, Abrahamowicz M, et al. Routine immunologic tests in systemic lupus erythematosus: is there a need for more studies? J Rheumatol 1996;23:1891–6.
16. C-Y Chen, H-M Tsen, L-C Chen, et al .Use of a new fluorescence immunoassay to detect anti-dsDNA antibodies is more correlated with disease activity and complement than the ELISA method in SLE patients .lupus-journal Lupus 2003;12, 266-273.
17. Hussain1 N, Jaffery G, S Hasnain. Serum Complement C3 and C4 levels in relation to diagnosis of lupus nephritis. Tropical Journal of Pharmaceutical Research 2008;7: 1117-21.
18. Sabry A, Sheashaa H, El-Husseini A, et al. Markers of inflammation and atherosclerosis in Egyptian patients with systemic lupus erythematosus. Nephrology, 2006, 11:329–335.
19. Pizarro S, Monarrez Espino J, Ruiz A, Jara LJ, Nava A, Riebeling-Navarro C: Soluble vascular cell adhesion molecule-1 indicates SLE disease activity and specific organ involvement. Rev Alerg Mex 2007, 54:189-195.
20. Zaccagni h,Fried j ,Cornell j et al. soluble adhesion molecule levels ,neurosychiatric lupus and lupus related damage .Front Biosci 2004;9:1654-9

9/3/2014