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Non-Traditional Laboratory Markers and Disease Activity in Patients with Systemic Lupus Erythematosus

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ABSTRACT

While the history and physical examination are most important in assessing systemic lupus erythematosus (SLE) disease activity; laboratory tests are helpful in organ systems that cannot be assessed clinically and can be used to correlate with the disease activity. The aim of the study was to assess the usefulness of some non-traditional lab markers versus the traditional lab markers in the SLE disease activity. A total of 28 SLE patients participated in the current study. Assessing SLE disease activity has been created using the SLE Disease Activity Index (SLEDAI). Two types of lab markers were measured; the first was the traditional lab markers (urea, creatinine, ALT, AST, ESR (1st hour), CRP, C3, C4, Albuminuria, ANA, Anti DNA) and the second was some non-traditional lab markers (granzyme B, fas ligand and neopterin). Six cases (21.4%) were Lupus without nephritis, while 22 cases (78.6%) were diagnosed as Lupus Nephritis. Nine cases (32.1%) were non-active disease, while 19 cases (67.9%) were in disease activity. The ESR levels were elevated in all the active disease cases (67.9%) and in only 3 cases (10.7%) of the non-active disease cases (P=0.000). The mean level of the granzyme B was 341.10±38.04 pg/ml in active disease patients. Its level increased 70 % during the disease activity as compared to that of the control group (P= 0.000). Concerning the mean level of the Fas Ligand, it was 505.05±201.63pg/ml in the active disease patients, while it was 220.44±205.46pg/ml in the non-active disease patients. Its level increased more than two and half folds in the active patients (P=0.000) as compared to that of the control group. Finally, the mean level of the neopterin was 26.31±7.52 nmol/L in the active disease patients. Its level increased more than eight folds in the active disease as compared to that of the control group (P=0.000). The levels of the non-traditional lab markers (granzyme B, fas ligand and neopterin) are sensitive and well correlated to the disease activity in SLE patients.

Keywords: Non-traditional lab markers, SLE activity

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INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that is confused with many disorders and can affect different organs (1). The incidence rates of SLE range from 1 to 10 per 100,000 person/year and the prevalence rates range from 20 to 70 per 100,000(2). The female: male ratio is 6-10:1, with the peak incidence between 15 and 40 years(3).

The disease is characterized by the production of autoantibodies which leads to immune complex deposition, inflammation, and finally , permanent organ damage(3).

Continued disease activity has been accepted as part of the natural history of SLE. The patients continue to have disease activity 10 years after diagnosis(4). Because no single measure can describe status in all SLE patients, standardized indices for assessing SLE disease activity have been created. The most common measures used include the Lupus Activity Index (LAI), the SLE Disease Activity Index (SLEDAI), the Systemic Lupus Activity Measure (SLAM),and the British Isles Lupus Assessment Group (BILAG)(5). The SLEDAI is the easiest assessment tool to use(6).

While the history and physical examination are most important in assessing disease activity, laboratory tests are helpful in organ systems that cannot be assessed clinically(3). In addition to the traditional lab markers, some non-traditional lab markers (granzyme B, fas ligand and neopterin) may be used in the SLE disease activity(3).

FAS/FAS ligand system is the main extrinsic pathway for the initiation of apoptosis in numerous cells and tissues(7). Fas and FasL play an important role in the pathogenesis of SLE. Their real value are used also as a predictor for disease activity (8).

Granzyme B is a serine protease found in the granules of cytotoxic lymphocytes and natural killer cells (NK). It is secreted by these cells to mediate apoptosis in target cells(9). Its level is strongly related with the severity of SLE(10) .

Neopterin is a marker of macrophage activation(11). The increased level of neopterin may suggest an attempt of the patients' macrophage system to remove the apoptotic cell excess. Since the serum level of neopterin correlates with the overall lupus disease activity, it may be regarded as an index of SLE disease activity(12). It is a sensitive marker of the SLE disease activity(13).

This study assessed the usefulness of some non-traditional markers compared with the traditional laboratory markers in the SLE disease activity.

Patients and methods

Our study included 28 patients with SLE recruited from the Rheumatology Clinic, New Children Hospital, Cairo University. Their mean ages were 13 ±3.7 years, ranging from 5.5 to 18 years. Assessing SLE disease activity have been created using the SLEDAI(5). 28 healthy individuals matched for age and gender were used as control groups to measure the normal values of the non-traditional lab markers. Informed consent was obtained from the parents of the children according to the guidelines of the ethical committee of NRC, Dokki, Egypt.

All patients were subjected to the following lab markers:

1- Traditional laboratory markers: urea (mg/dL), creatinine (mg/dL), ALT (U/L), AST (U/L), ESR (1st hour), C-reactive protein (CRP), C3, C4, albuminuria, ANA and Anti DNA.

2- Some non-traditional markers: granzyme B (pg/ml), fas ligand and neopterin (nmol/L).Serum levels of fas ligand(pg/ml) semi quantitative measurement was done by in vitro ELISA Kit (Ray Biotech, Inc., USA) (8).Granzyme B in NK cell subsets were analyzed by flow cytometry(14). Neopterin measurement was done by using ELISA Kit (IBL International GmbH, Flughafenstr, 52 A, D-22335 Hamburg, Germany)(15).

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The data were collected and studied using SPSS 20 statistical program. The mean, standard deviation (SD), minimum, maximum and range were calculated for all quantitative variables. The quantitative data were examined by Kolmogrov Smirnov test for normality.

Qui square (X^2) test was used to correlate between the disease activity and the traditional lab markers.

The mean ± SD was calculate for the non-traditional lab markers of the control group. Then, test value of each marker was taken. Then, one sample t test was taken to compare each of the non-traditional lab markers of the SLE patients with its test value. Level of significance was considered at P-value < 0.05.

The percentage of increase of the non-traditional markers was calculated according to the following formula

$$Percentage\ of\ increase = \frac{\text{mean difference value (SLE value} - \text{Control value)}}{Control\ value}$$

RESULTS

A total of 28 cases with SLE (18 females and 10 males) participated in the current study. Their mean ages were 13 ±3.7 years, ranging from 5.5 to 18 years. Six cases (21.4%) were Lupus without nephritis, while 22 cases (78.6%) were diagnosed as Lupus Nephritis (14.3%, 28.6%, 17.9%, 17.9% grades I, II, III and IV respectively). Nine patients (32.1%) were in a non-active disease, while 19 (67.9%) were in disease activity (Table 1).

Table (1): Descriptive data of the active and non-active cases

			Grade of nephritis					Total
			Non nephritis	Grade I	Grade II	Grade III	Grade IV	
Activity	No activity	Count	1	0	4	3	1	9
		% of Total	3.6%	0.0%	14.3%	10.7%	3.6%	32.1%
	Activity	Count	5	4	4	2	4	19
		% of Total	17.9%	14.3%	14.3%	7.1%	14.3%	67.9%
Total		Count	6	4	8	5	5	28
		% of Total	21.4%	14.3%	28.6%	17.9%	17.9%	100.0%

The mean level of the ESR was 49.931 ±37.34, ranging 8-150. Their levels were 67.05 ±33.05 and 13.7813 ± 9.09 in the active and non-active SLE patients respectively. The ESR levels were elevated in all the active disease cases (67.9%) and in only 3 cases (10.7%) of the non-active disease patients (P=0.000) (Tables 2, 3).

Table (2): Lab data of the studied population

	N	Range	Minimum	Maximum	Mean	SD
Urea (mg/dL)	28.00	101.40	8.60	110.00	30.91	22.64
Creatinine (mg/dL)	28.00	77.60	.40	78.00	3.84	14.64
ALT (U/L)	28.00	54.00	6.00	60.00	18.64	10.74
AST (U/L)	28.00	31.00	7.00	38.00	24.00	7.01
ESR (1st hour)	28.00	142.00	8.00	150.00	49.93	37.34
Granzyme B (pg/ml)	28.00	218.30	181.70	400.00	297.74	72.14
Fas Ligand (pg/ml)	28.00	651.00	100.00	751.00	413.57	240.69
Neopterin (nmol/L)	28.00	37.00	3.00	40.00	19.05	12.38

The levels of C3 and C4 decreased in 9 and 7 SLE active disease patients (32.1%, 25%) respectively. Their levels also decreased in 2 cases (7.1%) of the non-active disease patients (P>0.05). The CRP level was negative in 8 /19 (28.6% of total) of the active disease patients (P<0.05) (Table 3).

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Table (3) Correlation between the disease activity and traditional lab markers

Category		Disease activity		Total	Significance
		Non active	Active		
C3	Decreased	2 (7.1%)	9 (32.1%)	11 (39.3%)	0.1
	Normal	7 (25.0%)	10 (35.7%)	17 (60.7%)	
C4	Decreased	2 (7.1%)	7 (25%)	9 (32.1%)	0.3
	Normal	7 (25.0%)	12 (42.9%)	19 (67.9%)	
Albuminuria	Absent	5(17.9%)	10(35.7%)	15 (53.6%)	0.6
	Present	4 (14.3%)	9 (32.1%)	13 (46.4%)	
ANA	Negative	7 (25%)	8 (28.6%)	15 (53.6%)	0.08
	Positive	2 (7.1%)	11 (39.3%)	13 (46.4%)	
Anti DNA	Negative	6 (21.4%)	10(35.7%)	16 (57.1%)	0.3
	Positive	3 (10.7%)	9 (32.1%)	12 (42.9%)	
CRP	Positive	9 (32.1%)	11 (39.3%)	20 (71.4%)	0.02*
	Negative	0 (0.0%)	8 (28.6%)	8 (28.6%)	
ESR	Normal	6 (21.4%)	3 (10.7%)	9 (32.1%)	0.000*
	Elevated	0 (0.0 %)	19 (67.9%)	19 (67.9%)	

* Statistically significant using X^2

The ANA and anti DNA were positive in 11 and 9 SLE active disease patients (39.3%, 32.1%) respectively. They were also positive in 2 and 3 cases (7.1%, 10.7%) of the non-active disease patients (P>0.05) (Table 3).

The mean level of the granzyme B in the SLE patients was 294.74 ±72.14 pg/ml, ranging from 181.70 to 400.00pg/ml. Its level was 341.10±38.04 pg/ml in the active disease patients, while it was 206.18±20.31 pg/ml in the non-active disease patients. Its level increased 3 % (p=0.3) in the non-active patients as compared to that of the control group. In addition, its level increased 70 % in the active disease patients as compared to that of the control group (P= 0.000) (Tables 2, 4).

Table (4) Comparison between disease activity and non-traditional lab. markers

Category	Disease activity	Percentage of increase	Mean± SD	SE	Mean difference	Test value	Significance
Granzyme B (pg/ml)	Non active	3 %	206.18±20.31	6.77	6.13	200	0.3
	Active	70 %	341.10±38.04	8.72	141.10		0.000*
Fas Ligand (pg/ml)	Non active	58 %	220.44±205.46	68.49	81.44	139	0.2
	Active	263%	505.05±201.63	46.26	366.05		0.000*
Neopterin (nmol/L)	Non active	32 %	3.72±0.67	0.22	0.92	2.8	0.000*
	Active	839 %	26.31±7.52	1.72	23.51		0.003*

* Statistically significant

Concerning the mean level of the Fas Ligand in the SLE patients, it was 413.57 ±240.69 pg/ml, ranging from 100.00 to 651.00 pg/ml. The level was 505.05±201.63 pg/ml in the SLE active disease patients, while in the non-active disease one it was 220.44±205.46 pg/ml. The level increased by 58% (p=0.2) during the non-active disease as compared to that of the control group. The level increased more than two and half folds in the active disease as compared to that of the control group (P=0.000) (Tables 2, 4).

Concerning the mean level of the neopterin in the SLE patients, it was 19.05 ±12.36 nmol/L, ranging from 3.00 to 40.00. Its level was 3.72±0.67 nmol/L in the non-active disease patients, and 26.31±7.52nmol/L in the active disease patients. Its level increased 32% in non-active disease (p=0.000) as compared to that of the control group. The level increased more than eight folds in the active disease as compared to that of the control group (P=0.000) (Tables 2, 4).

No significant correlation between the non-traditional and traditional lab markers was observed whether in active or non-active disease state (table 5).

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Table (5) Correlation between the different non-traditional and traditional markers

Activity			Granzyme B (pg/ml)	Fas Ligand	Neopterin (nmol/L)
Non-active disease	Urea (mg/dL)	Pearson Correlation	.629	.190	-.340-
		Sig. (2-tailed)	.070	.624	.370
	Creatinine (mg/dL)	Pearson Correlation	.203	.645	-.459-
		Sig. (2-tailed)	.600	.061	.214
	ALT (U/L)	Pearson Correlation	-.478-	-.095-	.165
		Sig. (2-tailed)	.193	.807	.672
	AST (U/L)	Pearson Correlation	-.134-	-.245-	.164
		Sig. (2-tailed)	.731	.526	.673
	ESR (1st hour)	Pearson Correlation	-.089-	-.069-	-.042-
		Sig. (2-tailed)	.820	.861	.914
	Granzyme B (pg/ml)	Pearson Correlation	1	-.280-	-.614-
		Sig. (2-tailed)		.466	.078
	Fas Ligand	Pearson Correlation	-.280-	1	-.150-
		Sig. (2-tailed)	.466		.700
	Neopterin (nmol/L)	Pearson Correlation	-.614-	-.150-	1
		Sig. (2-tailed)	.078	.700	
Active disease	Urea (mg/dL)	Pearson Correlation	.180	-.363-	.024
		Sig. (2-tailed)	.461	.127	.922
	Creatinine (mg/dL)	Pearson Correlation	.093	-.169-	.073
		Sig. (2-tailed)	.705	.488	.766
	ALT (U/L)	Pearson Correlation	.188	.224	.369
		Sig. (2-tailed)	.440	.356	.120
	AST (U/L)	Pearson Correlation	.041	.393	-.058-
		Sig. (2-tailed)	.868	.096	.814
	ESR (1st hour)	Pearson Correlation	.126	-.004-	.223
		Sig. (2-tailed)	.607	.988	.359
	Granzyme B (pg/ml)	Pearson Correlation	1	-.081-	.258
		Sig. (2-tailed)		.743	.285
	Fas Ligand	Pearson Correlation	-.081-	1	.135
		Sig. (2-tailed)	.743		.582
	Neopterin (nmol/L)	Pearson Correlation	.258	.135	1
		Sig. (2-tailed)	.285	.582	

DISCUSSION

The complex nature of SLE demands a meticulously derived history ,thorough physical examination, and appropriate laboratory analysis (3). In the current study 22 cases (78.6%) were diagnosed as Lupus Nephritis. 17/28 (60%) belonged to class I-III; whereas, 5/28 (17.9%) were of class IV. Non-of the current cases were class V, and VI. Classes IV-VI are associated with poor prognosis and decreased survival (16, 17).

In the present study 9/28 patients (32.1%) were in the non-active disease form, while 19/28 (67.9%) were in disease activity. It is well-known that patients continue to have disease activity 10 years after diagnosis(4).

The ESR levels were elevated in all active disease cases (19/28; 67.9%); however, their levels were elevated in only 3/28 cases (10.7%) of the non-active disease form. The CRP levels were negative in 8/28 (28.6%) of the active disease patients. The ESR and the CRP are markers of inflammation, but they do not accurately reflect disease activity(3). The ESR may be elevated in hypoalbuminemia , anemia (3) or renal insufficiency(18).

Autoantibodies lead to the formation of immune complexes, which consume complement. Hence, measuring levels of C3 and C4 may be helpful in the routine monitoring of SLE patients (3). The levels of C3 and C4 in the present study decreased in 9 and 7 active disease patients (32.1%, 25%) respectively. Their levels also decreased in 2 cases (7.1%) of the non-active disease patients (P>0.05). It seems that the decrease of C3 or C4

doesn't predict the disease activity. Their levels may be reduced with hematologic and renal flares on the same day the flare occurred(19).

The hallmark of SLE is the presence of antinuclear antibodies(3). Their production is the immunopathologic basis of the disease(3). The ANA and anti DNA in the current study were positive in 11 and 9 of the SLE active disease patients (39.3%, 32.1%) respectively. They were also positive in 2 and 3 cases (7.1%, 10.7%) of the non-active disease patients (P>0.05). Both ANA and anti DNA do not correlate well with disease activity(20). However, some authors stated that the levels of anti–double-stranded DNA antibodies tend to reflect disease activity, but not in all patients(21). A positive ANA is the most important finding to establish the disease initially (3). Many authors found the antibodies to double-stranded DNA in 40-60% of the SLE patients (20) which is higher than that of the present study. This difference might be related to the relatively small number of the studied patients in the present study. In the present study, we tested three non-traditional lab markers: granzyme B, fas ligand and neopterin.

The level of Fas Ligand expression on the lymphocyte in the current study increased depending on the activity of the disease. The level was 505.05±201.58pg/ml in the active disease patients, while in the non-active disease one it was 220.44±205.46pg/ml. The level increased one and half fold during the disease activity as compared to that of the non-disease activity. The level significantly increased more than two and half folds in the active disease patients as compared to that of the control group. Many authors reported the increase level of Fas Ligand with the disease activity(8). In addition, the elevated level of the Fas Ligand during the disease activity is correlated to leucopenia, and tissue and organ damage (22).

The mean level of the inflammatory marker neopterin was 3.72±0.67nmol/L in the non-active disease patients, and 26.31±7.52nmol/L in the active disease patients. Its level increased 32% in non-active disease; whereas, its level increased more than eight folds in the active disease patients (P=0.000). The increased level of neopterin may suggest an attempt of the patients' macrophage system to remove the apoptotic cell excess(12). Many authors stated that the neopterin level appeared to be clinically useful for isolated assessment of disease activity(23). It is a useful independent index for disease activity (24). Some authors added that the serum neopterin is more sensitive markers of disease activity than C3 or C4(13).

The mean level Granzyme B was 341.10±38.04 pg/ml in the active disease patients, while it was 206.18±20.31 pg/ml in the non-active disease patients. Its level increased 3 % in the non-active disease patients; whereas, its level increased 70 % in the active disease patients. The granzyme B is found in the granules of cytotoxic lymphocytes and natural killer cells (NK). It is secreted by these cells to mediate apoptosis in target cells(9). The higher level of the soluble granzyme B in SLE patients was reported by many authors who added that its level is strongly related to the severity of SLE (10). The increased level observed during the disease activity in the present study may be attributed to the phenotypic and functional changes in the NK cells during the disease activity as many authors stated (14). Another possible explanation to the elevated granzyme level during the disease activity is the reduction of apoptotic bodies' clearance from phagocytic/macrophage system resulting in an increased apoptotic burdens that results in hyper activation of the immune system cells(25).

In conclusion, the levels of the non-traditional lab markers (granzyme B, fas ligand and neopterin) are sensitive and well correlated to the disease activity in SLE patients. There was also no significant correlation between these non-traditional markers and the traditional markers. So we suggest using these non-traditional markers as routine lab investigation to prove and assess the disease activity in SLE patients with elevated or positive traditional lab markers (ESR, CRP, C3, C4, ANA, and Anti DNA).

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