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Relation between Lymphopenia and Internal Organ Involvement in Systemic Lupus Erythematosus Patients

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease, characterized by autoantibody production and immunocomplex formation, leading to widespread inflammatory damage involving multi-organ systems. Lymphopenia is a common laboratory involvement seen in patients with SLE and the mechanism of it is still unclear.

Objectives: The aim of the current study was to investigate the relation between lymphopenia and clinical manifestations, laboratory findings, and disease activity in systemic lupus erythematosus (SLE) patients.

Patients and Methods: It was a cross sectional study; with a total of 60 patients with SLE recruited from the Rheumatology and Rehabilitation outpatient clinic at Sohag University Hospital. Demographic data, personal history, detailed history of general health condition and chronic or current diseases were reported. All the participants were subjected to detection of erythrocyte sedimentation rate, liver function tests, renal function tests, complete blood count (CBC), renal biopsy, protein/creatinine ratio and/or 24hr protein in urine, urine analysis, ANA profile, and Complement 3 and 4.

Results: Two thirds of the study population had normal lymphocytic count, and one third had lymphopenia. Lymphopenia group showed significantly more hypochromic anemia with significant lower hemoglobin level and lower MCV. The mean creatinine level was significantly higher among lymphopenic cases. Lymphopenic cases had higher proteinuria.

Conclusions: It could be concluded that **l**ymphopenia in patients with SLE may be used as indicator of renal involvement in these patients.

Keywords: Systemic lupus erythematosus, Lymphopenia, Renal involvement.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease, which is characterized by excess autoantibody production and immunocomplex formation, leading to widespread inflammatory damage involving multi-organ systems. It may affect any organ and produce a broad spectrum of clinical manifestations [(1)]. Lymphopenia is a common laboratory involvement seen in patients with SLE and the mechanism of it is still unclear [(2)].

The clinical usefulness of lymphopenia has been limited mainly to aid in lupus diagnosis because lymphopenia is one of the hematologic criteria according to the American College of Rheumatology (ACR). Lymphopenia was found in the majority of SLE patients (over 60%) on initial diagnosis and this rises to over 90% during the disease course of SLE [(3)].

Lymphopenia was seen to be correlated with disease activity in adults with SLE. However, it may be caused by multi-factors other than SLE itself [(4)].

Medications including corticosteroids, cytotoxic agents (e.g. cyclophosphamide) and many infections may contribute to the reduction in lymphocyte count [(5)]. Some studies have shown lymphopenia to be associated with particular clinical manifestations of SLE, disease activity and organ damage [(1, 6)].

The aim of this study was to investigate the relation between lymphopenia and clinical manifestations, laboratory findings, disease activity in SLE patients.

PATIENTS AND METHODS

This cross-sectional study included 60 patients classified as SLE according to either the 2012 SLICC criteria or the new 2019 ACR/EULAR SLE classification criteria. Patients were recruited from the Rheumatology and Rehabilitation outpatient clinic at Sohag University Hospital.

Inclusion criteria:

1- Patients diagnosed as SLE according to SLICC 2012 or ACR/EULAR 2017 classification criteria.
2- Age (18-60) years
3- Patient with disease duration more than 6 months

Exclusion criteria:

1- Other autoimmune disease including rheumatoid arthritis, scleroderma, mixed connective tissue disease and polymyositis.
2- Patients who had active infections, malignancies, hematologic diseases, hepatosplenic diseases.

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All patients were evaluated as follow:

1- Full history taking (demographic data and personal history, detailed history of general health condition and chronic or current diseases).
2- Full clinical examination including: General examination and vital signs, and complete rheumatological examination.
3- Routine investigations (erythrocyte sedimentation rate, liver function tests, renal function tests)
4- ANA by immunofluorescence.
5- ANA profile for the most common 19 autoantibodies by immunoblot.
6- All the participants were subjected to detection of: Complete Blood count (CBC), protein creatinine (P/C) ratio and/or 24hr protein in urine, urine analysis, complement 3, 4 and renal biopsy.

Ethical approval:

An approval of the study was obtained from Sohag University Academic and Ethical Committee. Each participant was informed about the research objective and methods in detail and using simple language prior to being requested to provide written informed consent prior to participation in 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.

Statistical analysis

Statistical package for social sciences (IBM-SPSS), version 25 IBM- Chicago, USA (August 2017) was used for statistical data analysis. Data expressed as mean, standard deviation (SD), number and percentage. Mean and standard deviation were used as descriptive value for quantitative data, while number and percentage were used to describe qualitative data. Student t test was used to compare the means between two groups, and Mann Whitney test was used instead of Student t test in case of non-parametric data. Pearson Chi square was used to compare percentages of qualitative data, and Fisher's Exact test was used for non-parametric data. P value < 0.05 was considered significant.

RESULTS

The patients were classified according to presence of lymphopenia into two groups; non-lymphopenic group (n=40, 66.7), and lymphopenic (n=20, 33.3%). The mean age of the study population was around 32-33 years, with no significant difference between the two groups. All of the cases were females. The mean disease duration of the study groups was 4±4.7 years for cases with lymphopenia compared to 3.6±4 years for those with normal lymphocytic count, with no significant difference.

Table (1): Demographic and basic clinical data of the study population

Group	Lymphopenia (N=20)	No Lymphopenia (N=40)	P value
Mean age (years)	32.95±7.37	32.10±10.19	0.741
Disease duration	3.99±4.71	3.59±3.97	0.740

There was non-significant difference between the two groups as regards azathioprine, cyclophosphamide and hydroxychloroquine therapy. However, cases with normal lymphocytic count received significantly higher doses of steroids compared to those with lymphopenia and only 3 cases needed mycophenolate mofetil treatment (Table 2).

Table (2): Comparison of drug history in the study groups

	Lympho-penia positive (n=20)	Lympho-penia negative (n=40)	P value
Azathioprine	11 (55%)	25 (62.5%)	0.402
Hydroxy-chloroquine	12 (60%)	26 (65%)	0.441
Steroids	5 (25%)	26 (65%)	**0.019***
Cyclo-phosphamide	8 (40%)	22 (55%)	0.419
Myco-phenolate mofetil	1 (5%)	2 (5%)	1.0

*P value < 0.05 was considered significant

Regarding ANA; the majority of cases had ANA titres between 1/80 to 1/320, with no significant difference between the two groups regarding ANA titre (P= 0.818) (Figure 1).

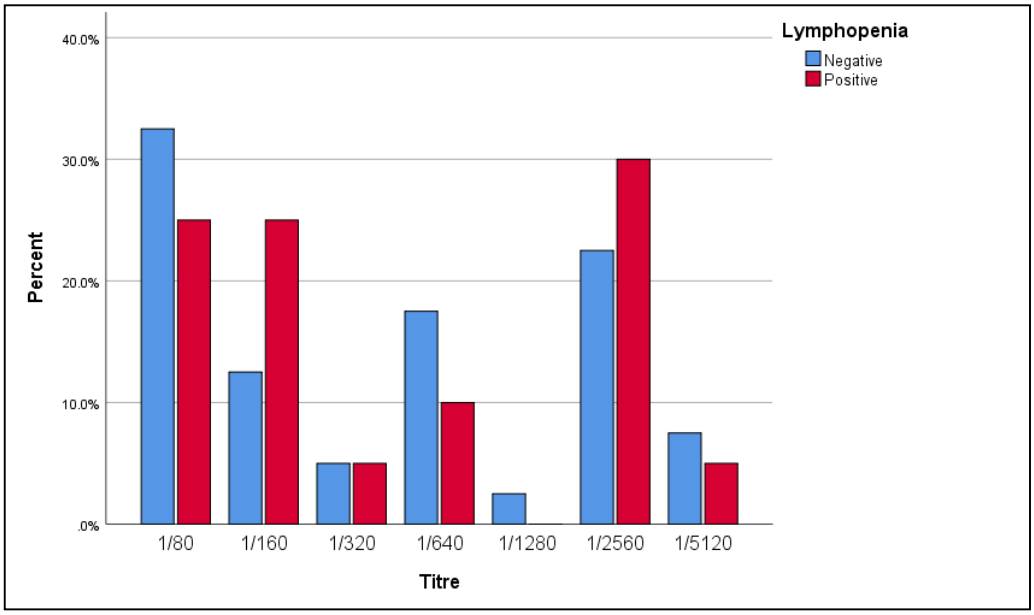

Figure (1): ANA titre in the study groups

Homogenous pattern was the dominant pattern among lymphopenic cases (12, 60%), followed by speckled pattern (5, 25%). In patients with normal lymphocytic count; the dominant pattern was the speckled one (20, 50%), followed by homogenous pattern (14, 35%). There was no significant difference between the two groups as regards ANA patterns (P= 0.214).

The most common ANA antibodies were the anti dsDNA, anti-Smith, anti-nucleosome, anti Sm-RNB; followed by anti-Ro and Anti La. All these antibodies showed non-significant differences between the two groups (Table 3).

Table (3): ANA profile in the study groups

	Lymphopenia		Chi square	P value
	Positive (n=20)	Negative (n=40)		
Anti ds- DNA	10(50%)	27(67.5%)	1.727	0.189(NS)
Anti Smith	6(30%)	11(27.5%)	0.041	0.839(NS)
Anti-nucleosome	7(35%)	19(47.5%)	0.849	0.357(NS)
Anti Ro-52	5(25%)	5(12.5%)	1.500	0.278(NS)
Anti Ro-60	5(25%)	9(22.5%)	0.047	1.000(NS)
Anti La	1(5%)	0	2.034	0.333(NS)
Anti CENP	1(5%)	0	2.034	0.333(NS)
Anti histone	2(10%)	4(10%)	0.000	1.000(NS)
Anti Sm-RNP	5(25%)	11(27.5%)	0.043	1.000(NS)
Anti ribosome	1(5%)	1(2.5%)	0.259	1.000(NS)
Anti U1-RNB	2(10%)	8(20%)	0.960	0.471(NS)
Anti Jo-1	0	1(2.5%)	0.508	1.000(NS)
Anti PCNA	0	1(2.5%)	0.508	1.000(NS)
Anti SCL-100	0	2(5%)	1.034	0.548(NS)
Anti Ku	0	2(5%)	1.034	0.548(NS)

The group of lymphopenia showed significantly more liability to develop complement deficiency (both C3 and C4). Other elements of the 2019 ACR/EULAR classification criteria for SLE showed non-significant differences between the two groups (Table 4).

Table (4): 2019 ACR/EULAR criteria for SLE among both groups

		Lymphopenia		Chi square	P value
		Positive (n=20)	Negative (n=40)		
Fever		14(70%)	23(57.5%)	0.881	0.348(NS)
Mucocutaneous	Alopecia	0	1(2.5%)	0.508	1.000(NS)
	Oral ulcers	14(70%)	25(62.5%)	0.330	0.566(NS)
	Subacute cut. lupus	0	0	-	-
	Acute cut. lupus	1(5%)	4(10%)	0.436	0.656(NS)
Musculoskeletal	Arthritis	10(50%)	17(42.5%)	0.303	0.582(NS)
Neurological	Delirium	0	0	-	-
	Psychosis	2(10%)	3(7.5%)	0.109	1.000(NS)
	Seizures	1(5%)	1(2.5%)	0.259	1.000(NS)
Serositis	Pleural or pericardial effusion	1(5%)	5(12.5%)	0.833	0.653(NS)
	Pericarditis	0	0	-	-
Hematological	Leucopenia	4(20%)	10(25%)	0.186	0.756(NS)
	Thrombo-cytopenia	7(35%)	4(10%)	5.566	**0.031(S)**
	Hemolytic anemia	0	2(5%)	1.034	0.548(NS)
Renal	Isolated proteinuria	0	1(2.5%)	0.508	1.000(NS)
	Class II or V LN	3(15%)	8(20%)	0.233	0.736(NS)
	Class III or IV LN	1(5%)	4(10%)	0.436	0.656(NS)
Anti-phospholipid	APS	1(5%)	4(10%)	0.436	0.656(NS)
Complement	C3 or C4	2(10%)	7(17.5%)	0.588	0.443(NS)
	C3 and C4	5(25%)	1(2.5%)	7.500	**0.013(S)**
Specific SLE antibodies	Anti ds- DNA	10(50%)	27(67.5%)	1.727	0.189(NS)
	Anti Smith	6(30%)	11(27.5%)	0.041	0.839(NS)

The mean total score of the 2019 ACR/EULAR criteria in the lymphopenic group was 15.60±5.91, while in non-lymphopenic group was 15.88±4.54, with no significant difference between the two groups (P value= 0.842).
The lymphopenia group showed significantly more hypochromic anemia with significant lower hemoglobin level and lower mean corpuscular volume (MCV). The mean creatinine level was significantly higher among lymphopenic cases compared to those with normal lymphocytic count. The P/C ratio was higher among cases with lymphopenic cases compared to those with normal lymphocytic count, but with a non-significant difference (Table 5).

Table (5): Laboratory investigations in the study groups

	Lymphopenia	Mean	Std. Deviation	T test	P value
WBCs ($x10^3$/ul)	Positive	7.367	1.937	0.657	0.514
	Negative	6.521	1.908		(NS)
Lymphocytes %	Positive	15.04%	3.41%	9.319	**<0.001**
	Negative	41.78%	9.43%		**(HS)**
HB (g/dl)	Positive	9.125	1.614	3.020	**0.004 (S)**
	Negative	10.521	1.713		
MCV (fL)	Positive	72.685	8.411	3.028	**0.004 (S)**
	Negative	79.411	7.843		
PLT ($x10^3$/ul)	Positive	218.710	8.233	1.693	0.096 (NS)
	Negative	264.821	17.912		
ESR (mm/hr.)	Positive	70.63	8.092	1.008	0.318
	Negative	62.03	3.261		
ALT (U/L)	Positive	22.90	5.106	336*	0.386
	Negative	24.59	5.323		
AST (U/L)	Positive	36.05	8.368	289*	0.105
	Negative	24.56	6.885		
Creatinine (mg/dl)	Positive	1.142	0.080	2.717	**0.009 (S)**
	Negative	0.766	0.153		
Protein/creat ratio	Positive	1.236	0.14	0.265	0.792 (NS)
	Negative	1.104	0.17		

* Mann Whitney test was used instead of t test due to non parametric data-Lymphopenic cases had higher proteinuria compared to cases with normal lymphocytic count, with a significant difference (P= 0.002) (Figure 2).

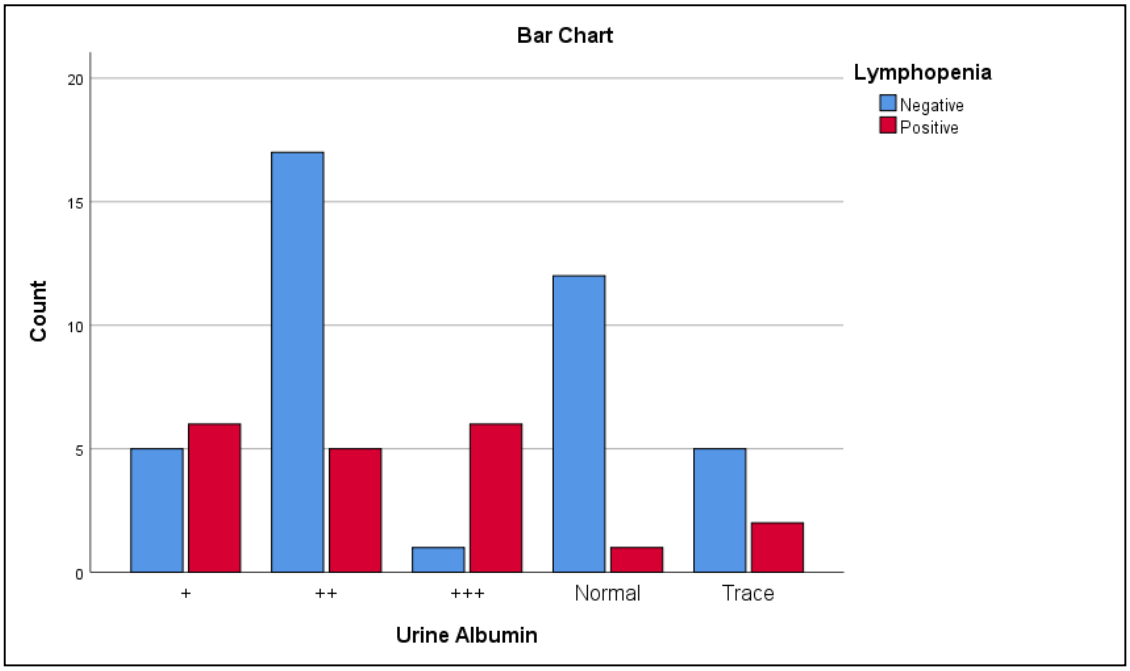

Figure (2): Urine albumin in the study groups

DISCUSSION

SLE is characterized by a wide variety of clinical and laboratory presentations affecting almost all organ systems and may results in severe organ or life-threatening manifestations [(7)].

Lymphopenia is a common clinical manifestation of SLE and is one of the hematological criteria according to the 1997-ACR classification criteria of SLE [(8)]. Lymphopenia has been reported in up to 90% of SLE patients along their disease course [(4)].

Two thirds of this study population had normal lymphocytic count, and one third had lymphopenia which is lower than study of **Sonhy *et al.*** [(9)] as lymphopenia was found in 46% of the their patients. Moreover, **Rivero *et al.*** [(10)] found a higher frequency of 78%.

The mean age of this study population was around 32-33 years, with no significant difference between the two groups. All of the cases were females. In our study, the mean disease duration of the study groups were 4 years for cases with lymphopenia compared to 3.6 years for those with normal lymphocytic count, with no significant difference.

In this study, there was non-significant difference between lymphopenia groups as regards azathioprine, cyclophosphamide and hydroxychloroquine therapy. However, cases with normal lymphocytic count received significantly higher doses of steroids compared to those with lymphopenia and only 3 cases needed mycophenolate mofetil treatment.

Also, in study of **Faddah *et al.*** [(11)], there were no statistically significant differences in drug intake including hydroxychloroquine, azathioprine, cyclophosphamide (IV pulse) and mycophenolate mofetil between lymphopenic and non-lymphopenic patients which is similar to present study. On the other hand, **Yu *et al.*** [(4)] found that lymphopenia was significantly related to pulse steroid therapy (methyl prednisolone 1 gm daily for 3-5 days) taken during lupus flares. Furthermore, **Vilá *et al.*** [(6)] found that lymphopenia was associated with both corticosteroids and azathioprine intake but not with hydroxychloroquine or pulse IV cyclophosphamide.

The majority of our cases had ANA titres between 1/80 to 1/320, with no significant difference between the two groups regarding ANA titre, homogenous pattern was the dominant pattern among lymphopenic cases, opposite to those with normal lymphocytic count, where the dominant pattern was the speckled one. However, the difference was non significant. The most common ANA antibodies were the anti dsDNA, anti-Smith, anti nucleosome, anti Sm-RNB; followed by anti Ro and Anti La. All these antibodies showed non significant differences between the two groups. In line with our results, **Faddah *et al.*** [(11)] found in their study that there were no significant differences between autoantibodies viz ANAs, anti-dsDNA, aCL (IgG and IgM) among lymphopenic and non-lymphopenic patients. In contrast, **Vilá *et al.*** [(6)] stated that lymphopenia was positively associated with anti-dsDNA antibodies but not with ANA or aCL. Also, **Yu *et al.*** [(4)] found that lymphopenia was significantly associated with anti-dsDNA antibodies. The explanation here may be the possible lymphocytotoxic activity of anti-dsDNA antibodies through cross-reactivity between nuclear antigen and lymphocyte membrane [(12)]. Contrarily, other authors didn't find any significant association with anti-ds DNA [(4, 11)]. This is in line with the present results.

Thrombocytopenia was significantly more common among cases with lymphopenia. The complement system is an essential part of the innate immunity system [(13)], as it plays an important role in the

removal of atypical antigens and immune complex [14]. In SLE, hypocomplementemia is an important serological marker of ongoing inflammation where complement elements are "consumed" by tissue bound immune complexes [15]. In spite of some few exceptions, a strong correlation was suggested between SLE disease activity especially renal flare and drop in complement levels (what is called C3 and C4 consumption) [16, 17]. Taking in consideration the association of lymphopenia with disease activity in the current study, the association between lymphopenia and consumed C3 is an expected result. This is supported by **Sobhy *et al.*** [9] and a previous study of **Yu *et al.*** [4] while on the other hand, others found no link [11].

In the current study, the lymphopenia group showed significantly more hypochromic anemia with significant lower hemoglobin level and lower MCV, the mean creatinine level was significantly higher among lymphopenic cases compared to those with normal lymphocytic count. Lymphopenic cases had higher proteinuria compared to cases with normal lymphocytic count, with a significant difference. The P/C ratio was higher among cases with lymphopenic cases compared to those with normal lymphocytic count, but with a non-significant difference. However, the results of **Faddah *et al.*** [11] showed that lymphopenia was significantly associated with leucopenia but not with hemolytic anemia or thrombocytopenia. In accordance to our study, **Vilá *et al.*** [4] **and Yu *et al.*** [6] both found that lymphopenia was associated with leucopenia.

In this study, the total score of the 2019 ACR/EULAR criteria had similar means between the two groups, with no significant difference. There was non-significant difference between the two groups regarding classes of lupus nephritis. The total number of biopsy-diagnosed lupus nephritis was limited in our study, and this may explain the non-significant difference between the two groups.

We found that the mean activity score of SLE was slightly higher among cases with lymphopenia compared to those with normal lymphocytic count, with non-significant difference. Similar to our study, **Sobhy *et al.*** [9] and **Vilá *et al.*** [6] found that the SLEDAI was higher in the lymphopenic SLE group compared to non lymphopenic SLE one, although their results did not reach the statistical significance threshold. On the other hand, **Faddah *et al.*** [11] reported that there was a statistically significant association between lymphopenia and disease activity which was also the results of both **Yu *et al.*** [4] and **Mirzayan *et al.*** [18]. This may be explained by the fact that in active SLE, lymphocytes may undergo apoptosis resulting from activation induced cell death through Fas and Fas ligand pathway [19, 20] or death by neglect-apoptosis pathway [21]. Also, CD4+ and CD8+ T-cells that bear the CD28 molecule may decrease in the peripheral blood of SLE patients. CD28 mediated costimulation influences T-cell susceptibility to activation induced cell death and may be involved in T-cell lymphopenia [19]. Also, anti-CD4 antibodies are frequently found in patients with SLE [22].

CONCLUSIONS

It could be concluded that lymphopenia in patients with SLE may be useful in prediction of internal organ involvement. Lymphopenia was associated with more hypochromic anemia with significant lower hemoglobin level and lower MCV, higher creatinine level, and higher proteinuria. The P/C ratio was higher among cases with lymphopenic cases compared to those with normal lymphocytic count, but with a non-significant difference.

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