Med. J. Cairo Univ., Vol. 88, No. 3, June: 1055-1063, 2020
www.medicaljournalofcairouniversity.net

CD5+ B Lymphocytes in Systemic Lupus Erythematosus Patients: Relation to Disease Activity

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Abstract

Background: Systemic Erythematosus (SLE) is a chronic, complicated and challenging disease to diagnose and treat. The etiology of SLE is unknown, but certain risk factors have been identified that lead to immune system dysregulation with pathogenic autoantibody formation and immune complex deposition.

Aim of Study: To assess blood concentration of CD5+ B cells in patients with SLE and to evaluate their relationship with SLE disease activity.

Patients and Methods: The present study included forty SLE patients who were selected from outpatient clinic of Rheumatology and Rehabilitation of Ain Shams University Hospital and diagnosed according to new EULAR and ACR classification criteria. Based on SLEDAI, the patients were selected and divided into two groups. The first group included 20 patients with inactive disease and the second group included 20 patients with active disease. They were matched with ten healthy individuals as a control group, and all were subjected to full history, clinical examination, ESR, CRP, serum complements, anti-dsDNA, ANA, serum creatinine twenty-four hours urinary proteins as well as CD5+ B lymphocytes by flow cytometric analysis.

Results: In the present study, the percentage of CD5+ B lymphocytes per total lymphocytes were significantly decreased in SLE patients compared to healthy individuals. Moreover, the percentage of CD5+ B lymphocytes per total B cells were significantly decreased in SLE patients compared to controls. We also have found a statistically highly significant decrease in the percentage of CD5+ B cells in active SLE patients compared to inactive patients. As regards the correlation studies, the results revealed a positive correlation between CD5+ B cells and each of platelets, C3 and C4. Moreover, the diagnostic performance of CD5+ B cells was evaluated and our results showed that CD5+ B cells can discriminate SLE patients from controls, and can predict the disease activity.

Conclusion: The proportions of CD5+ B cells were significantly decreased in SLE patients than normal people, and were significantly decreased in active SLE patients than inactive ones. These findings denote that CD5+ B cells may have a potential role in preventing autoimmunity development.

Key Words: *Systemic lupus erythematosus – CD5+ B cells – Disease activity.*

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Introduction

SYSTEMIC Lupus Erythematosus (SLE) is a prototypic autoimmune disease with diverse clinical manifestations in association to autoantibodies to components of the cell nucleus [1].

Generalized immune cell abnormalities that involve the B cell, T cell, and monocyte lineages were found in SLE patients [2]. These immune cell abnormalities appear to promote B cell hyperactivity which is responsible for the production of an array of autoantibodies with immune-complex deposition and tissue injury [3].

There are conflict data on B cell precursors that generate these autoantibodies. Indeed, numerous studies tried to localize or identify the cells that produce them in SLE patients [4-6].

Peripheral blood B lymphocytes are generally divided into distinct B cell subsets which differ in their cellular markers [7]. CD5+ B cells are a small population of B lymphocytes which exhibit unique developmental, phenotypic and functional characteristics that differ from the majority of B cells [8].

The role of CD5+ B cells in health and disease has long been a matter of debate. Although some would indicate that they are the source of these autoantibodies [9], recent evidence has shown that high-affinity antibodies to double-stranded DNA (dsDNA) in SLE and Rheumatoid Factor (RF) in

Rheumatoid Arthritis (RA) are derived from CD5-negative B cells and not from CD5+ B cells [10,11].

It has been shown that CD5 + B cells spontaneously secrete natural antibodies which serve in removal of apoptic cells, and counter pathogenic IgG [12]. They also secrete interleukin 10 which has a regulatory effect on different immune population [13]. Furthermore, as long as CD5 co-receptor is expressed on B lymphocytes, the threshold of B-cell receptor is elevated and the cells are maintained in anergy. Subsequently, proliferation and antibodies production are limited [14]. These recent findings denote that CD5+ B cells have a protective role against autoimmunity, and that their reduced level might predispose to autoimmunity development [15].

Aim of the work:

The aim of the study is to assess blood concentration of CD5+ B cells in patients with SLE and to evaluate their relationship with SLE disease activity.

Patients and Methods

This is a case-control study which was carried out at the outpatient clinic and the inpatient of Physical Medicine and Rheumatology Department, Ain Shams University Hospital, Cairo, Egypt; during the period from May 2018 to January 2019.

The study was approved by the Ethics Committee of the Faculty of Medicine at Ain Shams University. An informed verbal consent was obtained from patients and controls after detailed discussion with him/her to explain the aim and the steps of the study.

Study population:

Patients group: The study included forty SLE patients. Diagnosis of SLE was based on new EULAR and ACR Classification criteria for SLE [16].

Exclusion criteria: Patients with other associated autoimmune diseases e.g. Hashimoto's thyroiditis. Patients suffer from end-stage organ failure. Pregnant patients. Patients suffer from malignancy. Patients take Drug-Related Lupus (DRL) eg. Chloropromazine, hydralazine, isoniazid, methyldopa, momocycline, and procainamide.

Control group: 10 age and gender matched healthy individuals were randomly recruited to the study as control.

Study measurements:

All patients were subjected to the followings:

Clinical evaluation: Full history taking and complete clinical examination.

Laboratory investigations: CBC complete blood picture assayed by automated coulter. Erythrocyte Sedimentation Rate (ESR) by Westergren bolt method. C-reactive protein by latex method. Complement level (by radial immunodiffusion assay) C3, C4. Anti -double-stranded DNA antibodies (anti-dsDNA) by immune fluorescence. Anti-Nuclear Antibody (ANA) by immune florescence assay. Serum creatinine assayed on autoanalyzer Hitachi 917 and urea. Complete urine analysis. Twenty-four hours' urinary proteins.

Assay for CD5+ B Lymphocytes: By flow cytometric analysis using BECKMAN COULTER, France.

Flow cytometric analysis: Cells preparation and surface staining: Blood collection was performed in sterile Ethylene Diamine Tetra Acetate (EDTA)-filled blood collection tubes. One hundred microliter of blood was taken and added in Falcon tube with 20 gl of PerCp-conjugated anti-CD20 antibodies (clone: L27, BD) and 20 gl PE-conjugated anti-CD5 (clone: UCHT2, BD) and incubated for 20min at 4ºC. After surface staining, lysis of red blood cells was done by using 2ml of the lysis buffer (BD pharmingen) vortex to mix well and incubating for 10min at room temperature in the dark, followed by centrifugation for 5min at a speed of 1500rpm and discarding the supernatant. The pellets were washed twice with 2ml Phosphate Buffer Saline (PBS) then centrifuged for 5min at a speed of 1500rpm. Supernatant was removed and the pellet was resuspended in 500 gl of PBS for acquisition. The cells were acquired and analyzed by (BECKMAN COUTER, France). Lymphocytes were gated depending on both side and forward scatter. From the gated lymphocytes (A) Percentage of CD5+ B cells from total lymphocytes was calculated by using dot plot quadrants from the gated lymphocytes (A). CD5+ B cells were identified as double-positive cells for CD20 and CD5.

Assessment of disease activity: Disease activity was assessed using Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [17]. Items were recorded when the descriptor had been present at the time of the visit or in the preceding 10 days. Based on SLEDAI, patients were selected and divided into two groups. The first group included

20 patients with inactive disease (SLEDAI <4) and the second group included 20 patients with active disease (SLEDAI >4).

Statistical analysis:

The collected data was revised, coded, tabulated and introduced to a PC using statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

Descriptive statistics: Mean, Standard Deviation (± SD) and range for parametric numerical data, while median and Interquartile Range (IQR) for non-parametric numerical data. Frequency and percentage of non-numerical data.

Analytical statistics: Student's "*t*" test was used to assess the difference between two study group means. ANOVA test was used to assess the difference between more than two study group means. Correlation analysis: To assess the strength of association between two quantitative variables. The correlation coefficient defined the strength and direction of the linear relationship between two variables. The ROC Curve (receiver operating characteristic) provided a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. *p*-value: Level of significance: $p>0.05$: Non Significant (NS). $p<0.05$: Significant (S). $p<0.01$: Highly Significant (HS).

Results

Table (1): Demographic data in SLE patients and control group.

	Control group No.=10	Patients group No.=40	*p*-value	Sig.
Age:				
Mean ± SD	27.20±8.46	31.88±9.00	0.144	NS
Range	16-40	19-50		
Gender:				
Female	9 (90.0%)	36 (90.0%)	1.000	NS
Male	1 (10.0%)	4 (10.0%)		

Table (2): Clinical manifestations of SLE patients.

System affection	No. %
Constitutional	5 (12.50%)
Mucocutanous	18 (45.00%)
Musculoskeletal	15 (37.5%)
Neurology	1 (2.50%)
Lung affection	1 (2.50%)
Cardiac affection	1 (2.50%)

Table (3): Laboratory data among the studied groups.

	Control group No.=10	Patients group No.=40	*p*-value	Sig.
TLC ($X10^3/mm^3$):				
Mean ± SD	5.75±0.91	7.36±2.39	0.043	S
Range	4.5-7	3.5-10.8		
Hemoglobin (gm/dl):				
Mean ± SD	12.70±0.92	10.41±1.15	0.001	HS
Range	11.5-14.2	8.5-13		
Platelets ($X10^3$):				
Mean ± SD	273.5±61.47	232.13±63.82	0.071	NS
Range	190-360	99-330		
ESR (mm/hour):				
Mean ± SD	16.5±4.70	54.33±30.04	0.000	HS
Range	11.0-24.0	10-120		
CRP (mg/dl):				
Mean ± SD	3.3±1.5	10.18±4.31	0.041	S
Range	1.5-6.3	5-15		
Anti-ds DNA:				
No. %	0 (0.0%)	26 (65.0%)	0.000	HS
ANA:				
No. %	0.0 (0.0%)	40 (100.0%)	0.000	HS
C3 (mg/dl):				
Mean ± SD	125.55±12.64	84.65±26.12	0.000	HS
Range	100-140	50-130		
C4 (mg/dl):				
Mean ± SD	35.73±11.82	19.75±8.35	0.008	HS
Range	20-50	7-40		
Urea (mg/dl):				
Median (IQR)	17.12 (14.98-21.4)	34.24 (19.26-60.03)	0.003	HS
Range	14.98-25.54	20-112		
Creatinine (mg/dl):				
Median (IQR)	0.6 (0.5-0.6)	0.9 (0.6-1.4)	0.007	HS
Range	0.3-0.7	0.5-2.2		
24h urine protein (mg/dl):				
Median (IQR)	90 (80-95)	123.8 (100-1078)	0.006	HS
Range	89-100	100-2082		

TLC : Total Leukocyte Count. C3 : Complement 3.
Hgb : Hemoglobin. C4: Complement 4.
ESR : Erythrocyte Sedimentation Rate.
Anti-ds DNA : Anti double stranded DN.

Table (4): Proportions of lymphocytes in SLE patients and controls.

	Control group No.=10	Patients group No.=40	*p*-value	Sig.
Total lymphocytes %:				
Mean ± SD	27.43±263	21.01±5.92	0.007	HS
Range	22.9-39.0	12.2-31.2		
B lymphocytes %:				
Mean ± SD	20.1±2.5	10.5±5.7	0.000	HS
Range	17.6-22.6	4.8-16.2		
CD20/CD5 B cells (%) of B lymphocytes:				
Mean ± SD	39.00±10.5	30.7±9.3	0.018	S
Range	28.5-50.5	21.4-40		
CD20/CD5 B cells (%) of total lymphocytes:				
Mean ± SD	11.1±4.9	4.80±2.42	0.000	HS
Range	5.09-16	0.4-8.9		

Table (5): Renal biopsy from lupus nephritis patients (n=22).

Renal biopsy	N	%
Class II	5	23
Class III	7	32
Class IV	10	45

Table (6): SLEDAI in patient group.

SLEDAI	Patients group No.=40
Median (IQR)	3.5 (2-8)
Range	0.20

Table (7): Comparison between inactive group and active groups as regard demographic data.

	Inactive group No.=20	Active group No.=20	*p*-value	Sig.
Age:				
Mean ± SD	32.75±8.76	31.00±9.39	0.288	(>0.05)
Range	20-48	19-50		NS
Gender:				
Female	16 (80.0%)	20 (100.0%)	0.108	(>0.05)
Male	4 (20.0%)	0 (0.0%)		NS
Duration:				
Median (IQR)	3.5 (2-10)	6.00 (3-10.5)	0.541	(>0.05)
Range	1-20	1-15		NS

Table (8): Comparison between active and inactive groups as regard system affection.

	Inactive group		Active group		*p*-value	Sig.
	No.	%	No.	%		
System affected	20	100.0	20	100.0		
Constitutional	0	0.0	5	25.0	0.017	(<0.05) S
Mucocutaneous	6	30	12	60	0.269	(>0.05) NS
Neurology	0	0.0	1	5.0	0.311	(>0.05) NS
Musculoskeletal	5	25.0	10	50.0	0.102	(>0.05) NS
Lung affection	0	0.0	1	5.0	0.311	(>0.05) NS
Cardiac affection	0	0.0	1	5.0	0.311	(>0.05) NS

Table (9): Comparison between active and inactive groups as regard laboratory data.

	Inactive group No.=20	Active group No.=20	*p*-value	Sig.
TLC (X1 0^{3}/mm^{3}):				
Mean ± SD	.31±1.95	6.41±2.45	0.005	(<0.01)
Range	4.5-10.8	3.5-10.5		HS
Hemoglobin (gm/dl):				
Mean ± SD	10.79±1.13	10.03±1.07	0.030	(<0.05)
Range	8.9-13	8.5-12.5		S
Platelets (X10^{3}):				
Mean ± SD	253.15±56.98	211.10±64.68	0.021	(<0.05)
Range	160-330	99-311		S
Urea (mg/dl):				
Median (IQR)	30.26	53.5	0.003	(<0.01)
Range	(17.12-25.68) 20-60.64	(40.5-86.5) 25-112		HS
Creatinine (mg/dl):				
Mean ± SD	0.88±0.28	1.33±0.43	0.000	(<0.01)
Range	0.5-1.1	0.6-2.2		HS
ESR (mm/hour):				
Mean ± SD	27.50±20.04	30.33±19.00	0.239	(>0.05)
Range	13-110	10-120		NS
CRP (mg/dl):				
Mean ± SD	8.10±1.02	9.05±2.05	0.055	(>0.05)
Range	5-10	6-15		NS
C3 (mg/dl):				
Mean ± SD	104.95±20.01	64.35±11.49	0.000	(<0.01)
Range	66-130	50-86		HS
C4 (mg/dl):				
Mean ± SD	26.60±4.75	12.90±4.66	0.000	(<0.01)
Range	20-40	7-25		HS
ANA:				
No. %	20 (100%)	20 (100%)	NA	NA
Anti-dsDNA:				
No. %	9 (45%)	17 (85%)	0.008	(<0.01) HS

TLC : Total Leukocyte Count.
ESR : Erythrocyte Sedimentation Rate.
Anti-ds DNA : Anti double stranded DN.
C3 : Complement 3.
C4 : Complement 4.

Table (10): Comparison between active and inactive groups as regard proportions of lymphocytes.

	Inactive group No.=20	Active group No.=20	*p*-value	Sig.
Lymphocytes %:				
Mean ± SD	23.45±5.61	18.57±5.29	0.004	<0.0 (HS)
Range	13-31.2	12.2-27.7		
B lymphocytes %:				
Mean ± SD	14.3±3.8	11.2±6.6	0.048	<0.05 (S)
Range	9.6-16.2	4.8-17.1		
CD20/CD5 B cells % of B Lymphocytes:				
Mean ± SD	31.7±11.2	23.5±10.8	0.024	<0.05 (S)
Range	21.4-42.9	18.1-40		
CD20/CD5 B cells % of total Lymphocytes:				
Mean ± SD	6.51±1.75	3.10±1.69	0.000	<0.01 (HS)
Range	2.8-8.9	0.4-5.8		

Table (11): Comparison between active and inactive groups as regard renal affection using chi-square test.

Renal affection	Inactive group		Active group		*p*-value	Sig.
	No.	%	No.	%		
Absent	8	40.0	10	50.0	0.069	>0.05 (NS)
Present	12	60.0	10	50.0		

Table (12): Comparison between active and inactive groups as regard classes of lupus nephritis using chi-square test.

Renal biopsy	Inactive group		Active group		*p*-value	Sig.
	No.	%	No.	%		
Class II	5	41.6	0	0.0	0.007	<0.01 (HS)
Class III	4	33.3	3	30.0		
Class IV	3	25.0	7	70.0		

Table (13): Comparison between active and inactive groups as regard SLEDAI.

SLEDAI	Inactive (No.=20)	Active (No.=20)	*p*-value	Sig.
Median (IQR)	2.00 (2-2)	8.00 (6-14)	0.000	(<0.01) HS
Range	0-3	4-20		

Table (14): Correlation between CD20/CD5 B cells % of total lymphocytes and age and disease duration.

	Cd20/cd5		
	r	*p*-value	Sig.
Age	–0.279	0.054	>0.05 (NS)
Duration	–0.229	0.062	>0.05 (NS)

Table (15): Relation between CD20/CD5 B cells % of total lymphocytes and gender of patients.

Gender	Cd20/cd5		*t*-test	*p*-value	Sig.
	Mean ± SD	Range			
Female	4.43±2.26	0.40-8.6	3.193	0.003	<0.01(HS)
Male	8.10±0.67	7.3-8.9			

Table (16): Relation between CD20/CD5 B cells % of total lymphocytes and system affection.

		Cd20/cd5		*t*-test	*p*-value	Sig.
		Mean ± SD	Range			
Musculoskeletal:	Negative	5.87±1.37	1.30-8.90	1.679	0.111	(>0.05) NS
	Positive	5.42±2.44	0.40-8.00			
Neurology:	Negative	4.91±2.35	0.40-8.90	1.806	0.051	(>0.05) NS
	Positive	1.60±0.00	1.60-1.60			
Mucocutaneous:	Negative	6.06±2.08	2.40-8.90	1.843	0.120	(>0.05) NS
	Positive	5.66±1.74	0.40-7.30			
Constitutional symptoms:	Negative	3.55±1.62	0.40-8.90	1.28 3	0.071	(>0.05) NS
	Positive	2.74±1.20	0.60-3.50			
Lung affection:	Negative	4.71±2.45	0.40-8.90	1.889	0.060	(>0.05) NS
	Positive	2.00±0.00	2.00-2.00			
Cardiac affection:	Negative	4.67±2.36	0.40-8.90	1.900	0.056	(>0.05) NS
	Positive	1.80±0.00	1.80-1.80			

Table (17): Correlation between CD20/CD5 B cells % of total lymphocytes and patient labs.

	Cd20/cd5		
	r	*p*-value	Sig.
TLC ($X10^3/mm^3$)	+ 0.200	0.071	>0.05 (NS)
Hemoglobin (gm/dl)	+0.262	0.057	>0.05 (NS)
Platelets ($X10^3$)	0.757**	0.000	<0.01 (HS)
Urea (mg/dl)	–0.073	0.209	>0.05 (NS)
Creatinine (mg/dl)	–0.171	0.181	>0.05 (NS)
ESR (mm/hour)	–0.054	0.242	>0.05 (NS)
CRP (mg/dl)	–0.231	0.060	>0.05 (NS)
C3 (mg/dl)	+0.748	0.000	<0.01 (HS)
C4 (mg/dl)	+0.723	0.000	<0.01 (HS)
Total lymphocytes %	+0.630	0.000	<0.01 (HS)
B lymphocytes %	+0.622	0.000	<0.01 (HS)

TLC: Total Leukocyte Count. C3: Complement 3.
ESR: Erythrocyte Sedimentation Rate. C4: Complement 4.

Table (18): Correlation between CD20/CD5 B cells % of total lymphocytes and Anti-ds DNA, ANA.

	Cd20/cd5		*t*-test	*p*-value	Sig.
	Mean ± SD	Range			
Anti-ds-DNA:					
Negative (n=14)	5.81±2.71	0.4-8.90	2.019	0.061	<0.05
Positive (n=26)	4.25±2.11	0.6-8			(NS)
ANA:					
Negative (n=0)	–	–	NA	NA	NA
Positive (n=40)	4.80±2.42	0.40-8.90			

Anti-ds DNA: Anti double stranded DNA.
ANA : Anti-nuclear antibody.

Table (19): Relation between CD20/CD5 B cells % of total lymphocytes and renal affection.

Renal affection	Cd20/cd5		*t*-test	*p*-value	Sig.
	Mean ± SD	Range			
Renal:					
Negative	4.26±2.13	0.60-8.90	2.099•	0.152	(>0.05)
Positive	4.75±2.03	0.40-8.60			NS

Table (20): Relation between CD20/CD5 B cells % of total lymphocytes and renal biopsy.

Renal biopsy	Cd20/cd5		*t*-test	*p*-value	Sig.
	Mean ± SD	Range			
Class II (n=5)	6.95±1.44	5.30-8.60	1.061	0.056	(>0.05)
Class III (n=7)	4.27±2.00	0.40-8.00			NS
Class IV (n=10)	4.01±.1.08	1.40-6.30			

Table (21): Correlation between CD20/CD5 B cells % of total lymphocytes and SLEDAI.

	Cd20/cd5		
	r	*p*-value	Sig.
SLEDAI	–0.727	0.000	<0.01(HS)

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index.

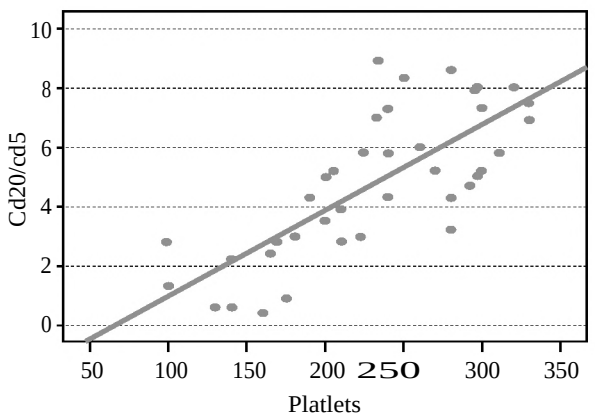

Fig. (1): Correlation between CD20/CD5 B cells of total lymphocytes and platelets.

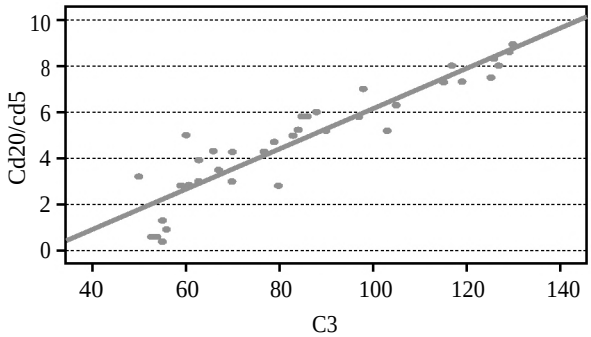

Fig. (2): Correlation between CD20/CD5 B cells of total lymphocytes and C3.

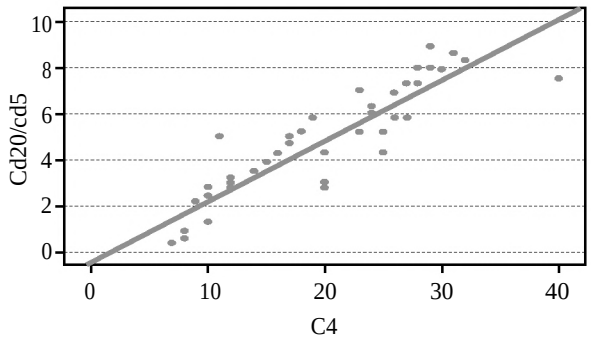

Fig. (3): Correlation between CD20/CD5 B cells of total lymphocytes and C4.

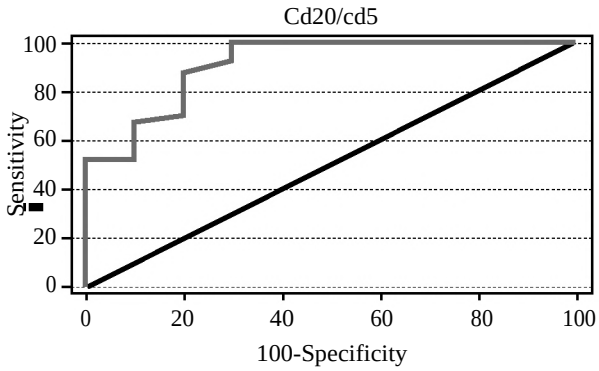

Fig. (4): Roc curve analysis showing the diagnostic performance of CD20/CD5 B cells to distinguish between SLE patients and controls.

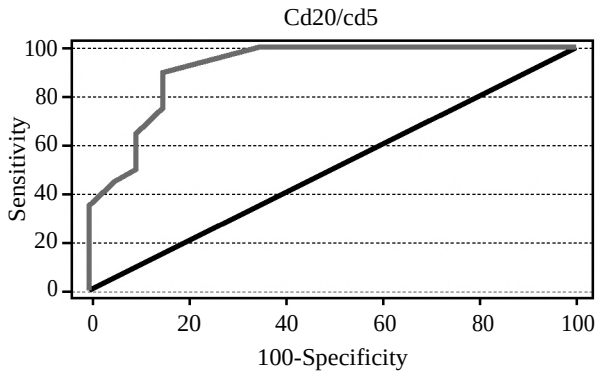

Fig. (5): Roc curve analysis showing the diagnostic performance of CD20/CD5 B cells to distinguish between active and inactive SLE patients.

Discussion

SLE is an idiopathic connective tissue disease with a spectrum covering wide array of clinical manifestations [1]. The etiology is multifactorial and B lymphocyte hyperactivity is thought to be one of these multiple factors [18]. Previously, it was believed that CD5+ B lymphocytes are the source of auto antibodies production and that their levels are increased in the blood of SLE patients [19]. Recent evidence has shown that CD5 negative B lymphocytes are the ones that produce these auto antibodies and not CD5+ B cells [20]. Recent studies have revealed that CD5+ B cells have a role in preventing autoimmunity by raising the threshold required for activation of self-reactive B cells [21]. Also, CD5+ B cells are shown to produce high levels of IL-10 and natural IgM with low affinity and poly-reactivity for autoantigens that have a role in removing auto-antigens and apoptotic cells [20]. Therefore, these regulatory B cells are considered as inhibitors of inflammation and autoimmune responses [10].

Whether CD5+ B lymphocytes increase or decrease in the blood of SLE patients is still a controversial issue which we tried to find out by comparing CD5+ B cells in 40 SLE patients and 10 healthy controls. The forty patients were divided into active and inactive groups. The two groups were compared as regard CD5+ B cells to assess its relation to disease activity.

As regard the proportion of lymphocytes in our patients, total lymphocytes percentage ranged from 12.2 to 31.2 with a mean of 21.01 ±5.92%. There was a highly statistically significant difference between patients and controls as (p=0.007) and between active and inactive ones (p=0.004). Our results are consistent with Amaylia et al., (2013) who reported that lymphopenia is common in SLE patients and it's a chief finding in lupus fluctuations reflecting case activity [22].

Percentage of total B lymphocytes were highly significantly decreased in our patients compared to controls as (p<0.01). Furthermore, we found a statistically significant difference between active and inactive patients as (p=0.048). Our results are consistent with Odendahl et al., (2000) who reported that SLE patients had significant B cell lymphopenia with some disturbances and impairments in all B cell types which are naïve, memory B cells and plasma cells [23].

We assessed the percentage of CD20+/CD5+ B lymphocytes per total B cells, and they were significantly decreased in SLE patients (30.7 ±9.3) % compared to controls (39.00 ± 10.5) % (p=0.018).

We also assessed the percentage of CD20+/ CD5+ B lymphocytes per total lymphocytes, and it was significantly decreased in SLE patients (4.80±2.42) % compared to healthy individuals (11.10±4.9) % (p<0.01).

These findings are consistent with the data obtained from Vernino L.A. et al (1992) who demonstrated that the percentage of CD5 + B cell was (24%) in ten normal subjects and it was about 17% in 16 SLE patients [24].

Unlike our findings, Markeljevic et al. (1994) measured the percentage of CD5-expressing B cells in peripheral blood of 28 SLE patients in the remission phase and found that relative to healthy control subjects, the blood CD5+ B cell subset tended to be elevated in SLE patients [25]. However, they did not measure the percentage of CD5-expressing B cells in active SLE patients which we think that it would add valued data about the proportion of CD5-expressing B cells in the entire SLE patients and not in patients in the remission phase only.

On the other hand, Böhm in 2004, conducted a study on 24 SLE patients which showed that SLE patients had increased percentages of CD5+ B cells compared to controls. However, patients included in his study were mainly cutaneous lupus patients [26]. The difference between our results and those reported by Böhm may be explained by the difference in the affected systems.

Garaud et al., (2009) reported that the percentage of CD5+ B cell was similar in both 25 healthy control and 25 SLE patients. However, they measured the membrane density of CD5 on B cells and it was lower in SLE patients than controls [27]. The decreased membrane density of CD5 on B cells in SLE goes in accordance with the new concept that membrane CD5 elevates the threshold of BCR mediated signaling and prevents B lymphocyte expansion and auto antibody production [18].

We reported a statistically highly significant decrease in the percentage of CD20/CD5+B cells per total lymphocytes in active SLE patients (3.10 ± 1.69) % compared to inactive patients (6.51 ±1.75) % (p<0.01). Our findings support that decreased CD5 B cells may have a role in disease activity.

In contrast to our results, Ebo et al. (1994) did not find any association between CD5+ B cells and SLE activity [28]. This difference between our

results and those reported might be explained by the difference in the criteria used for diseases activity categorization for SLE patients.

We also found a positive correlation between CD5+ B cells % of total lymphocytes and platelets (r=0.757 * *, p<0.01).

We have found a positive correlation between CD5+ B cells of total lymphocytes and each of C3 (r=0.748 * *, p<0.01) and C4 (r=0.723 * *, p<0.01). Our results are in agreement with Hassan et al., (2017) who reported a significant positive correlation between CD5 B cells of total lymphocytes and both of C3 and C4 **[29]**. The positive correlation with complement levels may be due to the role of complement receptors in selection, expansion, and maintenance of B-1 cells **[30]**. Also, B1 cells are positively selected in early development by cognate antigens and that interaction requires complement receptors.

Our results revealed a statistically highly significant correlation between CD20/CD5+ B cells of total lymphocytes and each of percentage of total lymphocytes and percentage of B lymphocytes as (r=0.630**, 0.622** respectively) (p<0.01) our results are different from results reported by Hassan et al., who did not find significant correlation between CD5+ B cells of total lymphocytes and any of them **[29]**.

We did not find a significant correlation between CD5+ B cells and age of patients, duration of the disease, ESR, CRP, other laboratory characteristics or system affection.

Conclusion:

In this study, CD5+ B cells were significantly decreased in SLE patients than control group, and highly significantly decreased in active SLE patients than inactive subgroup.

An important conclusion is that CD5+ B cells may have a potential role in preventing autoimmunity development and could be used as marker of SLE disease activity.

Thus, our results might be important as they suggest a novel approach to the clinical management of lupus patients.

Conflicts of interest: No conflict of interest.

Funding:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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الخلايا الليمفاوية B +CD5 فى مرض الذئبة الحمراء وعلاقتها بنشاط المرض

الذئبة الحمراء مرض مزمن يصعب تشخيصه وعلاجه، وبالرغم من كون سبب هذا المرض لا يزال مجهود، إلا آنه قد تم التعرف على بعض العوامل التى تؤدى إلى خلل فى تنظيم الجهاز المناعى وبالتالى تكوين الآجسام المضادة المسببة للمرض وترسب المركبات المناعية.

الهدف من الدراسة: إيجاد نسبة الخلايا الليمفاوية B +CD5 فى مرضى الذئبة الحمراء وتقييم مدى علاقتها بنشاط المرض.

المرضى وطرق البحث: شملت الدراسة آربعين مريضاً بالذئبة الحمراء تم إختيارهم من العيادة الخارجية التابعة لقسم الطب الطبيعى والروماتزم والتآهيل بجامعة عين شمس، وقد تم تشخيصهم وفقاً لمعايير التصنيف الجديدة الموصى بها من قبل الرابطة الآوروبية لمكافحة الروماتزم والكلية الآمريكية للآمراض الروماتزمية. وبإستخدام مؤشر نشاط مرض الذئبة الحمراء SLEDAI تم إختيار عشرين شخصاً ذو مرض نشط كمجموعة آولى بالإضافة إلى عشرين شخصاً ذو مرض نشط كمجموعة ثانية. كما ضمت الدراسة عشرة آفراد آصحاء تم إختيارهم كمجموعة ضابطة. وقد خضع جميع المشاركين فى الدراسة إلى آخذ بيانات عن تاريخهم المرضى مع الفحص الطبى والإختبارات المعملية مثل معدل سرعة ترسب كريات الدم الحمراء وإختبار البروتين المتفاعل وإختبا ر البروتينات المتممة ومضادات الحمض النووى وتحليل الآجسام المضادة للنواة والكرياتينين فى الدم وتحليل البروتين فى البول خلال ٢٤ ساعة بالإضافة إلى تحليل نسبة الخلايا الليمفاوية B +CD5 بواسطة تحليل التدفق الخلوى.

آظهرت النتائج: إنخفاض النسبة المئوية للخلايا الليمفاوية B +CD5 من إجمالى الخلايا الليمفاوية بشكل كبير فى مرضى الذئبة الحمراء مقارنة بالنسب المناظرة فى المجموعة الضابطة. كما إنخفضت آيضاً النسبة المئوية للخلايا الليمفاوية B +CD5 من إجمالى الخلايا الليمفاوية B بشكل كبير فى المرضى مقارنة بالمجموعة الضابطة. علاوة على ذلك، إنخفضت النسبة المئوية للخلايا الليمفاوية B +CD5 لدى مرضى الذئبة الحمراء النشطة بشكل كبير مقارنة بالمرضى غير النشيطين.

فيما يتعلق بدراسات الإرتباط، كشفت الدراسة عن وجود علاقة إيجابية بين نسبة الخلايا B +CD5 وكل من الصفائح الدموية والبروتينات المتممة C3 وC4. وقد تم تقييم الآداء التشخيصى لنسبة الخلايا الليمفاوية B +CD5 فى الدم وآظهرت النتائج آنه يمكن لنسبة الخلايا B +CD5 آن تميز بين مرضى الذئبة الحمراء والآفراد الآصحاء كما آنه يمكنها آن تقيم النشاط المرضى.

الإستنتاج والخلاصة: نستنتج من هذا آن الخلايا الليمفاوية B +CD5 قد يكون لها دور فى الحماية من الإصابة بالآمراض المناعية، وربما نستطيع إستخدامها كطريقة لتقييم مدى النشاط المرضى للذئبة الحمراء.