

Original Research Article

Role of Some Apoptotic Proteins in Pathogenesis of Juvenile Systemic Lupus Erytheromatosus and Lupus Nephritis

Abstract

Background: Apoptosis is increased in Systemic Lupus Erytheromatosus (SLE) patients with defective clearance of apoptotic cells, which lead to a pathological accumulation of abnormal cell debris and provide an autoimmune reaction. The aim of this work was to evaluate apoptosis and CD4/CD8 ratio in juvenile SLE patients and their relation to disease activity and lupus nephritis (LN).

Methods: This case-control study included 50 patients with jSLE divided into two groups according to SLEDAI score and twenty-five healthy children of matched age and sex as controls. All cases were subjected to detection of apoptosis, in the early stage through investigation of Annexin V and CD4/CD8 ratio through CD4 and CD8 T cells monoclonal antibody using the BD Accuri™ C6 Flow Cytometer Instrument.

Results: early apoptosis has statistically significant increase in the jSLE patients compared to controls with positive correlation to disease activity and no correlation to LN. (active group 20.77 ± 7.88 , inactive group 13 ± 6.8 and controls 3.53 ± 1.26). CD4/CD8 ratio has significant decrease in patients compared to controls with inversed correlation to disease activity and no correlation to LN. (active group 1.28 ± 0.51 , inactive group 1.6 ± 0.6 and controls 2.1 ± 0.6).

Conclusions: Apoptosis is high in jSLE patients with low CD4/CD8 ratio which related to disease activity, but not related to LN.

Keywords: Apoptotic Proteins, Pathogenesis, Juvenile Systemic Lupus Erytheromatosus, Lupus Nephritis.

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Please use official health descriptors Apoptosis Regulatory Proteins :[4ks]Comment

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change for imunology

Please put : Lupus Erytheromatosus, Systemic :[7ks]Comment

Introduction:

About five million people worldwide are afflicted with systemic lupus erythematosus (SLE), a chronic autoimmune inflammatory disease. It can impact practically all organ systems to varying degrees, including rashes, hair loss, arthralgias, glomerulonephritis, pericarditis, arthritis, pleuritis, psychosis, and seizures. The illness is distinguished by the generation of autoantibodies that target every immune system component ^[1].

Juvenile SLE(jSLE) or Childhood onset systemic lupus erythematosus (cSLE) is a form of SLE disease where symptoms begin before the age of 18 and usually more aggressive disease and worse outcome than the adult form ^[2].

Important phases in the pathogenesis of jSLE include apoptosis and elimination of apoptotic cells or materials. During apoptosis, altered nuclear debris is seen as alien and hazardous. To prevent the accumulation of apoptotic debris and the subsequent development of autoimmune reactions, it is necessary to eliminate apoptotic cells properly. Inducing a loss of lymphoid tissue tolerance, clearance deficits may be responsible for the development of autoimmunity. In the presence of autoantibodies, phagocytosis of apoptotic cells induces an inflammatory response. ^[3].

Immune system response and homeostasis are impacted by an imbalance between the functions of CD4+ and CD8+ T cells. CD4+ T-cells, also known as T helper (Th) cells, recruit and activate other immune cells, whereas CD8+ T-cells, also known as killer T-cells, create toxic granules that trigger apoptosis and cell death in pathogen-carrying cells. Recent evidence connects SLE and other autoimmune diseases to aberrant expression of CD4+ and CD8+ T cells ^[4].

The term Lupus nephritis (LN) means different patterns of renal injury in patients of SLE, through immune-mediated mechanism. The WHO gave classification of LN based completely on glomerular lesions in 1974, and subsequently underwent repeated transformations till the most accepted classification that was given by the International Society of Nephrology (ISN) and the Renal Pathology Society (RPS) ^[5].

Clinical signs of lupus nephritis are diverse, ranging from asymptomatic hematuria or proteinuria to nephritic and nephrotic syndromes, fast increasing glomerulonephritis, and chronic renal failure, all of which track the kidney's inflammatory process ^[6].

The purpose of this study was to quantify apoptotic proteins (Annexin V, CD8, CD4, and CD4/CD8 ratio) and evaluate their roles in the pathogenesis of jSLE in relation to disease activity and renal involvement.

Patients and Methods:

This case control study was carried out on 75 children attending Pediatric Nephrology Unit, Pediatric Department of Tanta University and the Immunogenetic division of National Research Center over a period of one year from June 2020 to May 2021. The study was done after being approved from the ethics committee, Faculty of Medicine, Tanta University (No. 31926/11/17). Informed consent was obtained from the children's parents or guardians.

Children were classified into equal groups: Group (1): 25 patients with active jSLE (SLEDAI \geq 4), group (2): 25 patients with inactive jSLE (SLEDAI $<$ 4) and group (3): 25 healthy controls with matched age and sex.

All children were subjected to: A full history was taken and complete clinical examination with laboratory investigations were done to all subjects. Full history including personal history (name, age, sex), family history, drug history, surgical history and menstrual history (if present). **General examination** including general appearance, Vital signs (temperature, blood pressure, heart rate and respiratory rate) and Anthropometric measures (weight and height). **Organ examination including skin lesions** (erythema, ulcerations, nodules or alopecia), mucous membranes (oral or nasal ulcerations), Musculo-skeletal examinations (any signs of arthritis: tender, swollen joint, limitation of mobility), neurological examinations including also visual and auditory examinations, respiratory examination, cardiac examination, abdominal examination, and extremities and lymph node examination. **Laboratory investigations:** Routine laboratory investigations (Inflammatory markers: CRP and ESR, Complete blood count (CBC): HB, TLC and PLTs, Renal functions: Urea, Creatinine., and proteins in 24hs, Liver functions: ALT, AST and albumin level). Immunological laboratory investigations [Complement levels: C3 and C4, Anti-nuclear antibody (ANA), Anti-DNA antibodies, Anti-Smith, Anti-RO, Anti-LA, Coomb's test, Lupus anticoagulant antibodies (LAC), Anticardiolipin antibodies (ACL) and Beta 2 glycoprotein antibodies (B2GP)]. **Renal biopsy** to determine the Pathology of LN and classify it according to International Society of Nephrology/Renal Pathology Society (ISN/PRS). (87) **Specific immunological lab tests** by flowcytometry: To ensure that the results were as typical of the in vivo setting as possible, all flow cytometry tests were completed within one hour of blood collection. **Early apoptosis (Annexin V Staining). CD4, CD8 and CD4/ CD8 ratio.**

In the early stage of apoptotic cells, the externalization of phosphatidylserine residues on the outer plasma membrane permits detection via Annexin V in tissues, and after the apoptotic cells are bound with FITC-labeled Annexin V, they may be detected using

fluorescence microscopy. In addition to sensitivity, the ability to confirm initiator caspase activity is a benefit. Negatively indicated are necrotic cell membranes. Therefore, demonstrating the membrane integrity of phosphatidylserine-positive cells is an essential test. Necrotic cells will stain with membrane-impermeable nucleic acid dyes, such as propidium iodide, since loss of membrane integrity is a pathognomonic sign of necrotic cell death^[7, 8].

Annexin V predominantly binds phosphatidylserine in the presence of protein-dependent Ca ion. Using flow cytometry and fluorescent dyes, it is possible to identify apoptotic cells^[7, 8]

Principle: It is used to determine the number of apoptotic cells. It will be recognised by staining the cells with Annexin V and propidium Iodide, followed by flow cytometric analysis. Normal cells are hydrophobic due to the presence of phosphatidyl serine in their inner membranes (side facing the cytoplasm). During apoptosis, the inner membrane flips to the outside membrane, exposing phosphatidyl serine. Annexin V recognizes the exposed phosphatidyl serine, whereas propidium iodide colors necrotic cells, which are distinguished from apoptotic cells by their leaking DNA content.^[9]

Reagents: FITC Annexin V, Propidium Iodide (PI), Lysing Buffer and 10X Annexin V Binding Buffer.

Procedure: 5 µl of FITC annexin V and 5 µl of PI were added to 100 µl of Whole blood. In a control tube, 100 µl of whole blood were added only. The tubes were incubated for 15 to 30 minutes in the dark. 1200 µl of lysing buffer were added and mixed well by vortex. For 8 minutes in the dark, the tubes were incubated. Centrifugation was done at 2000 rpm or 300 xg for 5 minutes. Supernatant was decanted and pellet was washed with 1 ml of 1X Annexin V Binding Buffer. Centrifugation was done at 1500 rpm or 200 xg for 5 minutes. Supernatant was decanted and pellet was resuspended in 400 µl 1X Annexin V Binding Buffer. Reading tubes and counting cells were done at the BD Accuri™ C6 Flow Cytometer Instrument.

CD4/ CD8 ratio: The typical CD4/CD8 ratio in healthy hosts is poorly characterised. In general, ratios between 1.5 to 2.5 are considered normal, although there is a great deal of variation because age, sex, genetics, ethnicity, environmental exposures, and illnesses may all alter the ratio^[10].

Variations in the CD4+/CD8+ ratio in healthy persons' peripheral blood may be symptomatic of immunodeficiency or autoimmune disorders. An inverted CD4+/CD8+ ratio

(less than 1/1) implies a weakened immune system owing to apoptotic or targeted cell death of circulating CD4 cells, CD8 cell expansion, or a combination of both processes [11].

Method for determining CD4 and CD8 T cells: CD4 and CD8 T cells were determined by FITC-conjugated anti-CD4 monoclonal antibody and FITC-conjugated anti-CD8 monoclonal antibody (BD Biosciences, United States) using the BD Accuri™ C6 Flow Cytometer Instrument.

Reagents: FITC-conjugated anti-CD4 monoclonal antibody, FITC-conjugated anti-CD8 monoclonal antibody, Lysing Buffer and PBS buffer.

Procedure: 5 µl of FITC anti-CD4 monoclonal antibody or anti-CD8 monoclonal antibody were added to 50µl of Whole blood. In a control tube, 50µl of whole blood were added only. Tubes were incubated for 15-30 minutes in the dark. 1 ml of lysing buffer was added and mixed well by vortex. Tubes were incubated for 8 minutes in the dark. Centrifugation was done at 2000 rpm or 300 xg for 5 minutes. Supernatant was decanted and pellet was washed with 1 ml of PBS. Centrifugation was done at 1500 rpm or 200 xg for 5 minutes. Supernatant was decanted and pellet was resuspended in 250 µl PBS. Reading tubes and counting cells were done at the BD Accuri™ C6 Flow Cytometer Instrument.

Statistical analysis

Version 20.0 of the IBM SPSS software suite was utilised to enter and analyse computer-entered data (Armonk, NY: IBM Corp). For qualitative data, numerical and percentage descriptors were given. To characterise quantitative data, we utilised the range (minimum and maximum), the mean, and the standard deviation. When the P value was less than 0.05, the findings' significance was evaluated. The Chi-square test is utilised to compare categorical data-based groupings. F-test (ANOVA) for variables with normally distributed data and Post Hoc test for pairwise comparisons. Using the Student's t-test for normally distributed quantitative data, two groups are compared. Evaluation of the link between two quantitative variables with irregular distributions using the Spearman correlation coefficient. The ROC curve graphically depicts the relationship between clinical sensitivity and specificity for each feasible test level. Additionally, the area under the ROC curve demonstrates the test's utility.

Results:

Demographic data of the studied children. Table 1

Table 1: comparison between the demographic data of the studied groups:

		Group1 (n=25)	Group2 (n=25)	Group3 (n=25)	F. test	p. value
Age (years)	Range	9 – 18	13 – 18	12 – 18	1.508	0.228
	Mean ± SD	15.94 ± 2.43	16.30 ± 1.55	15.36 ± 1.70		
Sex	Male (%)	3 (12.0%)	4 (16.0%)	3 (12.0%)	0.759	0.685
	Female (%)	22 (88.0%)	21 (84.0%)	22 (88.0%)		
	Male / Female ratio	1 / 7.3	1 / 5.3	1 / 7.3		
	Total M/F ratio	(1/6.1)				
Family H/O of SLE (%)		3 (12.0%)	3 (12.0%)		0.0	1.0
Weight (kg)	Range	28 – 59	46 – 59	42 – 58	0.757	0.473
	Mean ± SD	52.06 ± 7.03	53.44 ± 3.35	51.80 ± 4.04		
Height (cm)	Range	132-163.3	156 – 162.5	148 – 164	0.774	0.465
	Mean ± SD	158.71 ± 6.99	160.36 ± 1.81	159.30 ± 3.93		

F.test: ANOVA test, P. value: significant <0.05.

CRP and ESR (1st hour) showed significant increase in the patients groups compared to healthy controls and significant increase in active group compared to the inactive group. HB, TLCs and PLTs had a significant difference between the jSLE groups and the controls. Urea, Creatinine and 24 hour urinary proteins showed significant increase in the patients groups compared to controls with significant increase in the active compared to the inactive group. There was also a significant decrease in the serum albumin in the patients groups compared to the controls and significant decrease in the active compared to the inactive group. Table 2

Table 2: Comparison between routine laboratory investigations in the studied groups

Routine laboratory		Group1	Group2	Group3	F. test	P-value	Post Hoc	
CRP (mg/L)	Range	20 – 85	0 – 22	3 – 9	113.596	0.001*	P1	0.001*
	Mean ± SD	47.52± 19.31	4.68± 5.66	4.18 ± 1.94			P2	0.001*
							P3	0.880
ESR (mm/hr) 1st hour	Range	7 – 112	11 – 90	5 – 31	22.696	0.001*	P1	0.001*
	Mean ± SD	58.12± 18.81	35.64±12.62	17.08±7.35			P2	0.001*
							P3	0.003*
Hb K2(g/dl)	Range	8.7 – 13.2	8.6 – 13.2	10.7 – 13	3.109	0.048*	P1	0.661
	Mean ± SD	10.90± 1.36	11.05 ± 1.29	11.67±0.70			P2	0.022*
							P3	0.061
TLC (cell/mm3) × 103	Range	2.9 – 12	3.5 – 15.1	4 – 14	5.405	0.007*	P1	0.120
	Mean ± SD	5.92 ± 2.77	7.16± 2.52	8.50 ± 3.02			P2	0.002*
							P3	0.091
PLTs (Cell x 1,000/mm3)	Range	92 – 478	155 – 465	159 – 410	3.664	0.031*	P1	0.148
	Mean ± SD	211.12±100.54	245.88±63.16	275.32±83.93			P2	0.009*
							P3	0.219
Urea (mg/dL)	Range	12 – 56	8 – 59	6 – 18	16.621	0.001*	P1	0.017*
	Mean ± SD	28.38±12.65	21.24±11.93	11.56± 3.94			P2	0.001*
							P3	0.001*

Creatinine (mg/dL)	Range	0.4 – 1.9	0.2 – 1.2	0.4 – 1	5.328	0.007*	P1	0.003*
	Mean ± SD	0.94 ± 0.23	0.66± 0.23	0.71 ± 0.20			P2	0.014*
							P3	0.581
24hr urinary proteins (k2g/d)	Range	0.03– 3.6	0.01– 2.6	0.012_0.072	10.746	0.001*	P1	0.003*
	Mean ± SD	0.97 ± 1.15	0.35± 0.49	0.04 ± 0.02			P2	0.001*
							P3	0.132
ALT (IU/L)	Range	11 – 59	10 – 40	13 – 34	0.198	0.821	P1	0.581
	Mean ± SD	22.44 ± 9.79	21.20 ± 7.36	22.40± 6.14			P2	0.986
							P3	0.593
AST (IU/L)	Range	12 – 79	6 – 57	11 – 33	2.238	0.114	P1	0.346
	Mean ± SD	29.76 ± 17.76	26.24±13.19	21.92± 5.24			P2	0.052
							P3	0.248
Serum albumin (g/dl)	Range	1.1 – 4.8	2.9 – 5	3.3 – 5.2	13.831	0.001*	P1	0.001*
	Mean ± SD	3.09 ± 1.04	3.86 ± 0.54	4.20 ± 0.61			P2	0.001*
							P3	0.112

CRP: C- reactive protein, HB: Hemoglobin concentration, ALT: Alanine transaminase, ESR: Erythrocyte sedimentation rate, TLC: Total leucocytes count, AST: Aspartate transaminase, CBC: Complete blood picture, PLTs: Platelets, *: Statistically significant at $p < 0.05$, P1: indicate comparing between the active group and the inactive group, P2: indicate comparing between the active group and the control group, P3: indicate comparing between the inactive group and the control group.

ANA titre, Anti-ds DNA, ACL and LAC were positive in 94%, 90%, 34%, 24% respectively and had significantly increased the patients' groups compared to controls. On the other hand anti Smith ab, anti-RO, anti-LA, Coomb's test and B2GP ab were positive in 12%, 14%, 4%, 10% and 14% respectively and they did not show any significant difference. Regarding the C3 and C4 there was a significant decrease in the active jSLE patients groups compared to the inactive patients. Table 3

Table 3: Comparison between immunological laboratory investigations of the studied groups

Immunological labs	Group (1)	Group (2)	Group (3)	Total	X2	P-value
+ve ANA titre(n %)	25(100%)	22 (88.0%)	0(0%)	47 (94.0%)	63.271	0.001*
+veAnti-ds DNA (n %)	24 (96.0%)	21 (84.0%)	0(0%)	45 (90.0%)	57.000	0.001*
+ve Anti-Smith (n %)	4 (16.0%)	2 (8.0%)	0(0%)	6 (12%)	4.348	0.114
+ve Anti-Ro (n %)	4 (16.0%)	3 (12.0%)	0(0%)	7 (14%)	4.097	0.129
+ve Anti-La (n %)	1 (4.0%)	1 (4.0%)	0(0%)	2 (4%)	1.027	0.598
+ve Direct Coomb's (n %)	3 (12.0%)	2 (8.0%)	0(0%)	5 (10%)	3.000	0.223
+veACL (n %)	9(36.0%)	8(32.0%)	0(0%)	17(34%)	11.105	0.004*
+veB2GP (n %)	5(20.0%)	2(8.0%)	0(0%)	7(14%)	5.987	0.051
+ve LAC (n %)	7(28.0%)	5(20.0%)	0(0%)	12(24%)	7.738	0.021*
C3 (mg/dL)	Range	30 – 70	69 – 150	68 – 183	F: 69.679	0.001*
	Mean ± SD	39.4 ± 13.9	108.3 ± 26.4	122.9 ± 35.3		
	P1: 0.001*, P2: 0.001*, P3: 0.057					
C4 (mg/dL)	Range	7 – 20	19 – 72	18 – 50	F: 42.377	0.001*
	Mean ± SD	14.20 ± 3.79	36.8 ± 11.6	39.9 ± 10.4		
	P1: 0.001*, P2: 0.001*, P3: 0.136					

ANA titre: Antinuclear antibody titre, Anti-ds DNA: Anti-double stranded DNA antibody, ACL: Anti-cardiolipin antibody, B2Gp: Beta 2 glycoprotein antibody, LAC: Lupus anti-co agulant antibody, C4: Complement 4, C3: Complement 3.

Table 4 shows LN classes. LN class III and class IV were the commonest represented 41.9% and 29% respectively.

Table 4: Renal biopsy results of the studied jSLE patients

ISN/RPS classes of lupus nephritis	Group (1)	Group (2)	Total (n=31)
Class I	1 (4%)	0 (0%)	1 (3.2%)
Class II	2 (8%)	5 (20%)	7 (22.5%)
Class III	7 (28%)	6(24%)	13 (41.9%)
ClassIV	6 (24%)	3 (12%)	9 (29%)
Classv	1(4%)	0 (0%)	1 (3.2%)
X2		4.403	
P-value		0.355	

Table 5 and showed significant increase in the SLEDAI score in the active group ranging from 8 to 32 with Mean \pm SD (16.52 \pm 3.72).

Table 5: comparison of the SLEDAI scores between the active and inactive group

SLEDAI	Range	Group (1)	Group (2)	Test	P-value
	Mean \pm SD	8 – 32	16.52 \pm 3.72	0 – 2	T: 95.049

In early apoptosis there was statistically significant increase in the jSLE patients (active and inactive) compared to the controls with positive relation to disease activity. Regarding CD4 there was a significant decrease between the SLE patients (active group and inactive group) compared to the controls with inversed relation to disease activity. Regarding the CD8 there was no statistical significant difference between the entire studied groups. CD4/CD8 ratio had decreased significantly in the patients' groups compared to healthy controls with inversed relation to disease activity. Table 6

Table 6: Comparison between specific laboratory investigations of the studied groups

Specific lab		Group(1)	Group(2)	Group(3)	F. test	P-value		
Early apoptosis (% Annexin V-FITC Staining)	Range	8.4 – 35	4 – 31	0.9 – 6.7	34.321	0.001*	P1	0.001*
	Mean \pm SD	20.77 \pm 7.88	13.0 \pm 6.8	3.3 \pm 1.3			P2	0.001*
							P3	0.001*
CD4 (cells/mm3)	Range	7.5 – 35.7	12 – 38.6	19 – 46.7	33.643	0.001*	P1	0.001*
	Mean \pm SD	17.38 \pm 6.85	25.3 \pm 8.6	35.1 \pm 7.5			P2	0.001*
							P3	0.001*
CD8 (cells/ μ l)	Range	6 – 59	4.3 – 64	15 – 28.4	1.168	0.317	P1	0.206
	Mean \pm SD	22.12 \pm 11.14	18.4 \pm 13.3	22.4 \pm 4.2			P2	0.929
							P3	0.176
CD4/CD8 ratio (%)	Range	0.29 – 2.57	0.42 – 2.85	0.53 – 4.1	8.576	0.001*	P1	0.026*
	Mean \pm	1.28 \pm 0.51	1.6 \pm 00.6	2.1 \pm 0.6			P2	0.041*

	SD							
							P3	0.001*

CD4: cluster of differentiation 4, CD8: cluster of differentiation 8.

Figure 1 and 2 show: regarding the early apoptosis there was positive correlation with CRP and SLEDAI score and no correlation found for the other different studied parameters. Regarding CD4/CD8 ratio, there was a negative correlation with CRP, glucocorticoids dose and SLEDAI score with no statistically significant other correlations between CD4/CD8 ratio and the other studied parameters. There was no significant correlation for renal affection in form of (proteinuria, urea, creatinine) and early apoptosis or CD4/CD8 ratio.

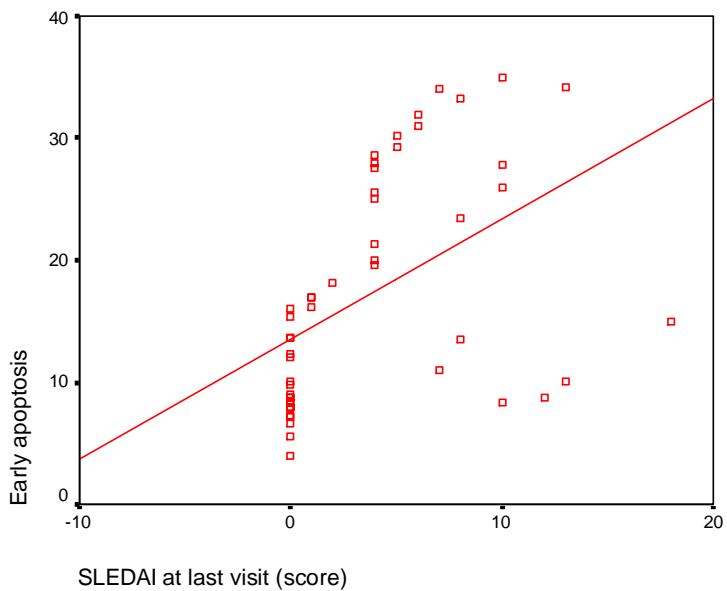


Figure 1: Significant positive correlation between the early apoptosis and the SLEDAI score of the studied jSLE patients

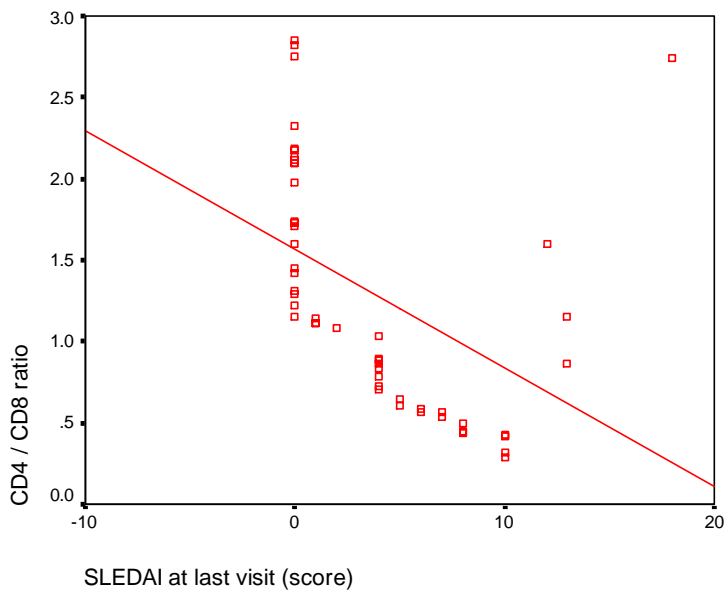


Figure 2: Significant negative correlation between the CD4/CD8 ratio and the SLEDAI score of the studied jSLE patients

Figure 3 show: in CD4/CD8 ratio, showed 76% sensitivity and 60% specificity with 68% accuracy. In early apoptosis, it showed 64% sensitivity and 60% specificity with 62% accuracy.

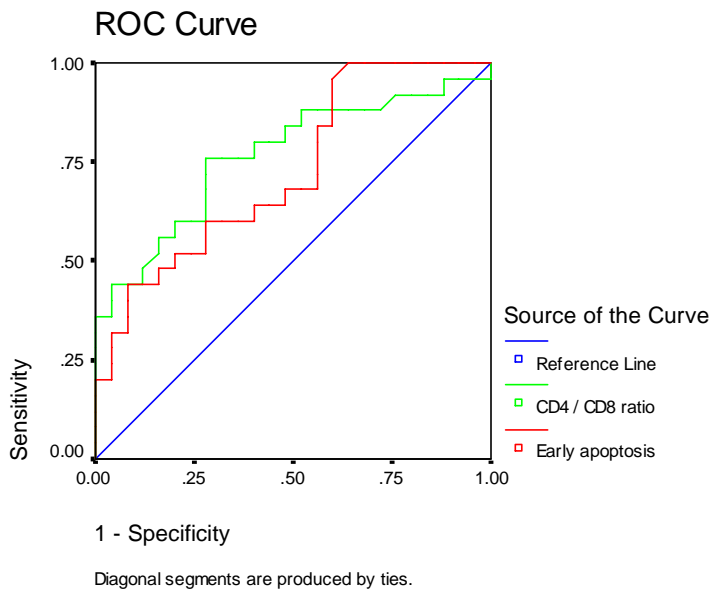


Figure 3: ROC curve for CD4/CD8 ratio and early apoptosis in the studied groups

Discussion

SLE pathogenesis has been related to dysregulated apoptosis as there is decreased clearance of apoptotic bodies, and increased death of lymphocytes, neutrophils, and macrophages(148),(149), auto-antigen exposure that can drive to an autoimmune response and combine with autoantibodies to form immune complexes^[12].

ANA and anti-ds-DNA antibody were positive in the patient groups in 94% and 90% respectively ,in agreement with this findings Qu et al. study^[13]. Anti-RO and Anti LA were positive in 14% and 4% respectivelywhile Anti-Smith was positive in 12%.These results are similar with Cozzani et al.study^[14]. ACL and the LAC had significant increase in the jSLE patients comparing to controls with percentage 34% and 24% respectively. Mohamed et al. got similar findings in his study with significantincrease in jSLE patients (ACL 28% and LAC 24%)^[15].

It is crucial to effectively monitor disease activity. Multiple indications of disease activity have been identified. All of these tools, however, have severe limitations that restrict their use, whereas the SLEDAI is the most often used marker of SLE disease activity ^[16]. SLEDAI score of the active patients was 16.52 ± 3.72 .our results agreed with Chiang et al. studyas in their study the mean SLEDAI score was 14.7 ^[17].

LN represented in 62% of cases agreed with our results a Dutch study, It includes111children with jSLE and 67/111 (60%) had LN^[18]. Proteinuria showed significant increase between the patient groups and the controls with positive relation to disease activity. This agreed withDuarte-García et al. study (158)and Mohamed et al. study.(159)Renal biopsy was done for the patients who had renal manifestations. Class III and class IV were the commonest type of LN 41.9% and 29% respectively. Same findings were found by Mavragani et al. study, they revealed about two third of the patients had LN classes III or IV^[19].

In the current study, the early apoptosis had increased significantly in the jSLE patients' groups comparing to the healthy controls with positive correlation to disease activity. In agreement with our study Courtney et al study ^[20], Su et al. study ^[21], Midgley et al.study^[22],Jin et al. study ^[23]and V et al. study^[24].

Also, early apoptosis was not significantly correlated with LN. Wang et al. found no statistically significant distinction between LN patients and healthy people ^[25].

In the current study, CD4 protein levels showed significant decrease in jSLE patients compared to controls with inversed significant correlation to disease activity.Agreed with our

results Lee et al. study^[26], Robinson et al. study^[27], Dal Ben et al study^[28] and Sonawale et al. study^[11].

CD8 protein expression was insignificant among the studied groups. In agreement with our results Sonawale et al. study^[23], but Against our results Robinson et al. study^[27] and Zabińska et al.^[29] study as their study revealed statistically significant increase in absolute count and percentage of CD8 in SLE patients compared to healthy controls with significant positive correlation between increasing absolute count of cells and disease activity.

The classification of SLE patients into two subpopulations, those with high or normal CD8 percentages and those with low CD8 percentages, is consistent with the CD8 results of Katsuyama et al. Eleven of thirteen patients with a high CD8 count had infectious episodes, whereas patients with a low CD8 count were never infected (0 out of 22 patients). It was shown that the number of CD8 T cells in the blood of SLE patients was independent of disease activity as defined by the SLEDAI, but positively correlated with infection rates^[30].

In the current study, CD4/CD8 ratio showed significant decrease between patients and controls with inversed significant correlation to disease activity. Agreed with our results Robinson et al.^[27], Sonawale et al. study^[11], Shah et al. study^[30] and Jiang et al. study^[31]

In the present study we found low CD4/CD8 ratio was not correlated with LN and against our results El-Girby et al.^[32] and Kopetschke et al study^[33].

Conclusions:

Significantly higher levels of apoptosis in jSLE patients compared to healthy controls, with a significant link to disease activity as determined by the SLEDAI score but no association with LN. In comparison to healthy controls, jSLE patients have significantly lower CD4 counts, whereas there is no significant difference in CD8 levels. Significantly lower CD4/CD8 ratio in jSLE patients compared to healthy controls, with an inverse link between disease activity as determined by the SLEDAI score and no association with lymph nodes.

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Please review the numbering of references. also review all references as number 32 is missing and is not correct.