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Circulating $CD4^+$ CD28 Null T Cells and $CD4^+$ $CD28^+$ T Lymphocytes in Systemic Lupus Erythematosis

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Abstract

Background: Systemic Lupus Erythematosis (SLE) is a chronic multi factorial systemic autoimmune disease which affects multiple organs such as joints, the skin, kidneys or central nervous system. T cells may be a contributing factor in pathogenesis of SLE.

Aim of Study: The objective of this study was to evaluate senescent $CD4^+$ CD28 null T lymphocytes and conventional $CD4^+$ $CD28^+$ T lymphocytes in SLE patients with and without nephritis.

Subjects and Methods: The study was conducted on 51 patients with SLE; 21 patients with nephritis and 30 patients without nephritis, in addition to 15 age and sex matched healthy individuals as control. The CD4/CD28 expression on peripheral blood lymphocytes was measured for all study subjects using flowcytometry technique.

Results: $CD4^+$/CD28 null T-lymphocytes showed no statistically significant difference among the studied groups. Meanwhile, $CD4^+$/$CD28^+$ T lymphocytes were significantly higher SLE patients with nephritis than controls (p=0.009). There was significant positive correlation between $CD4^+$/$CD28^+$ T lymphocyte population size and serum creatinine in SLE with nephritis (p=0.004).

Conclusion: $CD4^+$/$CD28^+$ T lymphocyte population was higher in SLE patients with nephritis than controls and show statistically significant changes in correlation with serum creatinine level.

Key Words: *$CD4^+$/$CD28^+$ – $CD4^+$/CD28null – Lupus – Nephritis – T-lymphocytes.*

Introduction

SYSTEMIC Lupus Erythematosus (SLE) is characterized by production of a wide range of autoantibodies that can induce inflammation associated with immunity in various tissues and organs [1]. It is believed that breakdown of immune endurance is one of the major mechanisms leading to the production of antibodies by B cells resulting in inflammation when linked to the self-antigens and consequent tissue damage [2]. Recent strong evidence has shown that T cells are indeed critical for the development of SLE, as they promote the autoantibodies production by providing significant support for B cells by stimulating them for differentiation, proliferation and maturation of self-antibodies expressed by B-cells [3]. Therefore, SLE is currently thought to be a T-cell controlled state. In fact, the molecules expressed on T-cells are targeted and their signaling pathways may be one of the possible therapeutic strategies in SLE.

Compared to healthy volunteers, a number of studies have shown that T cells isolated from SLE patients are abnormal in their phenomena and functions [4,5].

The aim of this study was to evaluate senescent CD4+/CD28 null and conventional $CD4^+$/$CD28^+$ T lymphocyte populations in patients with SLE with and without nephritis.

Subjects and Methods

This study was carried out, under ethical contributions, on SLE patients in the period from January 2015 to January 2018. The patients were recruited from Menoufiya University Hospital. The laboratory investigations were done in the Clinical Pathology Department, Faculty of Medicine. Ethics rules in the form of approval of the Ethics Committee of Menoufia University Hospitals. Informed consent was obtained from both patients and controls. The study involved 51 SLE patients; 21 SLE patients with nephritis (20 females, 1 male) with their ages ranging from 20 to 47 years

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old (mean ± SD 27.90±7.03) and 30 SLE patients without nephritis (29 females and 1 male) with their ages ranging from 20 to 48 years old (mean ± SD 32.07±8.96), and 15 apparently healthy individuals as a control group (all of them are females with their ages ranging from 23 to 48 years old (mean ± SD 32.93±7.61).

Patients were diagnosed according to clinical history taking and full physical examination, Systemic Lupus Erythematosis Disease Activity Index (SLEDAI) score, radiological investigations, routine laboratory tests including: Complete Blood Count (CBC), lipid profile, liver profile, Antinuclear Antibodies (ANA) and anti double stranded DNA (dsDNA) and high-sensitivity C-Reactive Protein (hs CRP) by turbidimetry. Renal biopsy was done for SLE with nephritis. For patients and controls, detection of expansion CD4/CD28 T lymphocyte populations was performed by flowcytometry.

Flowcytometric analysis: 1ml of EDTA blood sample was withdrawn.

A 100 µL of the washed suspension were stained with both monoclonal antibody antiCD28 (FITC) monoclonal antihuman antibodies (Biolegend, San Diego) and anti CD4 (PE) (Biolegend, San Diego) (5 µL of each monoclonal antibodies). After gentle mixing, cells were incubated for 20 minutes. Then the cells were washed twice with PBS, then the supernatant was discarded and the cells were re-suspended in residual buffer. Data were acquired on a FACS calibur flowcytometer (BD immunecytometry systems, San Jose, CA, USA) Fig. (1).

Statistical analysis:

Data collected were tabulated and analyzed by Statistical Package of Social Science (SPSS, Version 20; SPSS Inc., Chicago, Illinois, USA) on IBM personal computer. The following statistics were applied: Descriptive statistics: e.g. percentage (%), mean and Standard Deviation (SD) and analytic statistics: e.g. Chi-square test, student *t*-test, Mann-Whitney test, fisher's exact or monte Carlo correction, F-test (ANOVA), Kruskal Wallis test, Spearman coefficient. p-value <0.05 considered to be significant.

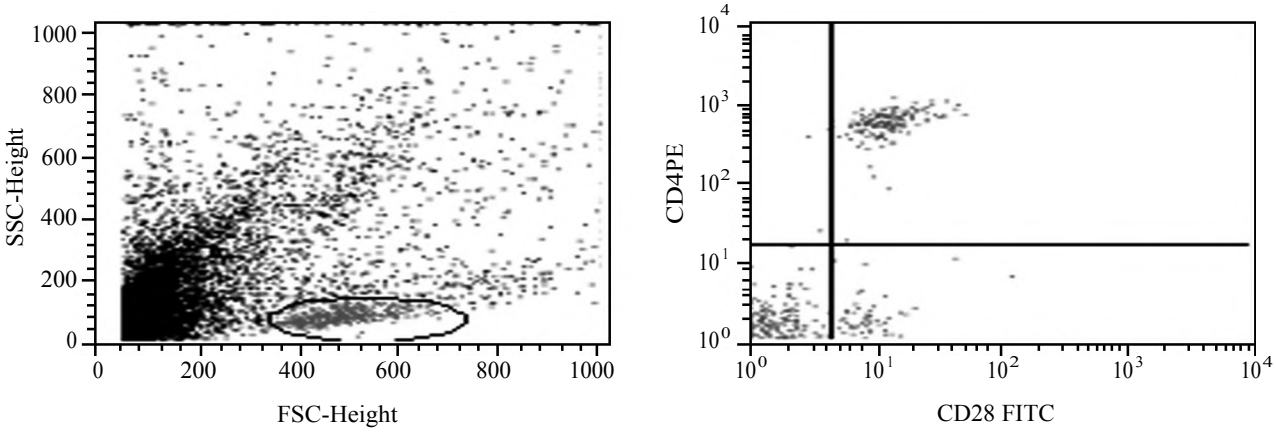

Fig. (1): CD4/CD28 expression on peripheral blood T lymphocytes from SLE patient with nephritis.

Results

The results of routine laboratory investigations are shown in (Table 1). Clinical diagnosis of SLE activity by Systemic Lupus Erythematosis Disease Activity Index (SLEDAI) score was shown in (Table 2). Comparison between the three studied groups; SLE with nephritis, SLE without nephritis and controls according to different T cell populations; CD4+/CD28null and $CD4^+/CD28^+$ T cells, revealed that $CD4^+/CD28^+$ T lymphocytes were significantly higher in SLE with nephritis than controls (p=0.009). There was no statistically significant difference in $CD4^+$/CD28 null T cell population among the 3 studied groups (p>0.05) (Table 3).

In SLE with nephritis group, logistic regression revealed that creatinine was the independent predictor for $CD4^+/CD28^+$ T lymphocyte population size (p=0.004) (Table 4).

Table (1): Comparison between the three studied groups according to laboratory parameters.

Items	SLE-nephritis (n=21)	SLE-non nephritis (n=30)	Control (n=15)	*p*	Significance between groups		
					p_1	p_2	p_3
Urea (mg/dl): Mean ± SD	54.86±37.87	19.37±3.08	18.73±4.01	<0.001*	<0.001 *	<0.001 *	0.653
Creatinine (mg/dl): Mean ± SD	1.80± 1.0	0.83±0.20	0.75±0.23	<0.001*	<0.001 *	<0.001 *	0.328
AST (U/L): Mean ± SD	49.29±34.01	23.40±3.32	23.13± 1.13	0.001 *	<0.001 *	0.007*	0.71
ALT (U/L): Mean ± SD	44.62±45.68	20.03± 1.99	21.53±0.92	<0.001*	<0.001 *	0.120	0.017*
Serum albumin (g/dl): Mean ± SD	3.02±0.80	3.93±0.59	3.76±0.40	<0.001*	<0.001 *	0.001 *	0.387
	(n=19)	(n=28)	(n=15)				
Serum calcium: Mean ± SD	8.77±0.92	8.75±0.26	8.75±0.31	0.984	–	–	–
Serum phosphorus (mg/dl): Mean ± SD	3.74±0.96	3.31±0.44	3.49±0.67	0.129	–	–	–
	(n=20)	(n=28)	(n=15)				
Total cholesterol (mg/dl): Mean ± SD	199.4± 19.0	192.0±31.22	170.9± 16.88	<0.001*	0.682	<0.001 *	<0.001 *
Triglycerides "TG" (mg/dl): Mean ± SD	156.2± 19.66	144.1± 18.18	104.5± 13.61	<0.001*	0.023 *	<0.001 *	<0.001 *
HGB (g/dl): Mean ± SD	9.73± 1.59	11.19± 1.46	12.97±0.72	<0.001 *			
WBCs (X10^3/µl): Mean ± SD	4.20± 1.58	5.06± 1.60	5.61±0.93	<0.001 *	–	–	–
Platelets (X10^3/µl): Mean ± SD	153.7±59.05	166.9±45.0	266.6±42.89	<0.001 *	–	–	–
INR: Mean ± SD	1.30±0.56	1.02±0.09	1.0±0.0	0.007*	–	–	–
ESR: Mean ± SD	81.05±36.19	46.87±48.49	7.80±2.57	<0.001 *	–	–	–
hs CRP: Mean ± SD	40.86±27.21	24.65±29.32	2.43±0.71	<0.001 *	–	–	–
Anti -dsDNA: Mean ± SD	35.57± 16.52	30.92±7.54	–	0.486	–	–	–

p1: *p*-value for comparing between SLE nephritis and SLE non nephritis. p_3: *p*-value for comparing between SLE non nephritis and control.
p2: *p*-value for comparing between SLE nephritis and control. * : Statistically significant at $p \leq 0.05$.

Table (2): Comparison between the two studied groups according to SLEDAI.

Items	SLE-nephritis (n=21)		SLE-non nephritis (n=30)		*p*
	No.	%	No.	%	
SLEDAI score:					
Mild (1-5)	2	9.5	6	20.0	0.402
Moderate (6-10)	3	14.3	7	23.3	
High activity (11-19)	12	57.1	10	33.3	
Very high activity >20	4	19.0	7	23.3	
Min.-max.	5.0-25.0		3.0-27.0		0.274
Mean ± SD	14.71±5.51		12.90±7.07		
Median	15.0		11.50		

Table (3): Comparison between the three studied groups regarding CD4 and CD28 expression.

Items	SLE-nephritis (n=21)	SLE-non nephritis (n=30)	Control (n=15)	*p*	Significance between groups		
					p_1	p_2	p_3
CD4+/CD28 null:							
Min.-max.	0.40-50.50	0.50-54.50	2.30-30.50	0.295	–	–	–
Mean ± SD	15.33± 13.28	12.61± 11.38	8.53±7.02				
CD4+/CD28+:							
Min.-max.	2.09-50.50	1.30-54.0	2.25-47.34	0.032*	0.077	0.009*	0.253
Mean ± SD	23.79± 14.03	30.52± 12.36	34.28± 11.69				

p1: *p*-value for comparing between SLE nephritis and SLE non nephritis.
p2: *p*-value for comparing between SLE nephritis and control.
p3: *p*-value for comparing between SLE non nephritis and control.
* : Statistically significant at $p \leq 0.05$.

Table (4): Multivariate linear regression for $CD4^+/CD28^+$ % T cells in SLE nephritis.

	B	SE	*t*	*p*
Creatinine (mg/dl)	–8.033	2.425	3.313*	0.004*
Platelets (X $10^3/\mu l$)	–1.241	1.480	0.838	0.413
Renal Biopsy	–3.158	1.851	1.707	0.106

r^2 = 0.729. F = 6.415*. p = 0.004*.

Discussion

SLE can affect the whole of body, but most often harmful organs are the skin, joints, heart, blood vessels, liver, kidneys, lungs and nervous system. The disease has variable course (remissions alternating with flares). Lupus can occur at any age, and is most common in females [6]. Nephritis is still one of the most critical complications of lupus with increasing incidence of end-stage renal disease [7]. Lupus Nephritis (LN) is the most common severe manifestation that affecting the majority of lupus patients. Even though there are a number of reports indicating that T cells play important roles in the pathogenesis of LN [8].

Many genetic factors, interactions of auto-antibodies, environmental invertebrates, T cells, B cell interactions, and immune defense processes interact to produce lupus erythematosus [9]. Patients with SLE develop pathogenic auto-antibodies that are targeted towards wide range of ubiquitous self antigens, including dsDNA and nuclear debris from apoptotic cells. These immune complexes deposit in organs, and the subsequent flow of inflammatory cells contributes to widespread tissue damage. Because of its multifactorial aetiology, the immunological pathogenesis of lupus nephritis is extremely complex and incompletely understood. However, it is generally recognized that loss of B cell tolerance associated with hyperactivity of CD4 T cells is central to pathogenesis of lupus [10].

Thus it was worthy to study CD4/CD28 T cells in patients with LN versus those without nephritis and controls aiming to understand an aspect of pathogenesis of LN.

The present study showed that the percentage of senescent CD4+ CD28 null T cells level in SLE patient's with nephritis was higher than lupus without nephritis then than controls but this didn't reach statistical significance (p>0.295).

Ugarte-Gil et al., [11] reported that circulating CD4+ CD28 null T cells is independently related to disease damage in systemic lupus erythematosus patients. Moreover, study by Broux et al., [12] showed that immune system aging contributes to increased morbidity and mortality in the elderly and may be premature in patients with disorders of immune system. One of the main characteristics of the immune effect is the expansion of CD4+ CD28 null T cells in the circulation. CD4+ CD28 null T cells have now been found to infiltrate target tissues of patients with rheumatoid arthritis, multiple sclerosis, acute coronary syndromes, myopathies and other immune-related diseases. Smaller number of subjects in our study should be put into consideration while interpreting our results.

Our study revealed that double $CD4^+/CD28^+$ T population was significant higher in patients with nephritis than controls (p=0.009). CD4+/ $CD28^+$ T population did not differ significantly between patients with and those without nephritis (p=0.077).

A study conducted by Mesquita, Jr. et al., [13] on CD4 T helper cells revealed that no difference in this population of cells between active lupus nephritis patients and lupus without nephritis. This could be considered consistent with our study.

Strickland et al., [14] study characterized an epigenetically altered CD4(+) $CD28(^+)$ T subset of cells in rheumatic autoimmune diseases and showed that the subset size is proportional to lupus flaring and severity. Some studies such as Driscoll et al., [15] expanded these reports by showing that activated genes in overexpressed experimental T cells on a subset of CD4+ $CD28^+$ T cells are present in active lupus patients, and that the size of this subset is directly proportional to the lupus activity (SLEDAI). However our study did not demonstrate significant correlation between $CD4^+/CD28^+$ T lymphocyte population and SLEDAI grades. The only parameter that was found to be independently positively correlated with $CD4^+/CD28^+$ population in SLE with nephritis was the serum creatinine level.

In Conclusion:

Increased percentages of circulating $CD4^+/CD28^+$ T lymphocytes cells that are positively correlated with creatinine was demonstrated in systemic lupus patients with nephritis.

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تقييم مجموعة الخلايا الليمفاوية الحاملة لسى دى ٤ وسى دى ٢٨ ومجموعة الخلايا الليمفاوية الحاملة لسى دى ٤ وغير حاملة لسى دى ٢٨ فى مرضى الذئبة الحمراء الجهازية

مرض الذئبة الحمراء الجهازية هو مرض مناعى ذاتى مزمن متعدد العوامل يؤثر على العديد من الآعضاء مثل المفاصل والجلد والكليتين والجهاز العصبى المركزى. كما إن الخلايا الليمفاوية تكون عاملا مساهما فى التسبب فى مرض الذئبة الحمراء.

تهدف هذه الدراسة إلى تقييم مجموعة الخلايا الليمفاوية الحاملة لسى دى ٤ وسى دى ٢٨ ومجموعة الخلايا الليمفاوية الحاملة لسى دى ٤ وغير حاملة لسى دى ٢٨ فى مرضى الذئبة الحمراء مع آو بدون إلتهاب الكلية.

آجريت الدراسة على ٥١ مريضا بمرض الذئبة الحمراء. ٢١ مريضا منهم يعانون من إلتهاب الكلية و٣٠ مريضا دون إلتهاب الكلية، بالإضافة إلى ١٥ من الآصحاء من نفس العمر والجنس. تم قياس تعبير السى دى ٤ والسى دى ٢٨ على الخلايا الليمفاوية فى الدم بإستخدام تقنية قياس التدفق الخلوى.

آظهرت النتائج الإحصائية آن الخلايا الليمفاوية الحاملة لسى دى ٤ وسى دى ٢٨ عند مرضى الذئبة الحمراء آعلى بكثير مع إلتهاب الكلية (p=0.009). كما آن هناك إرتباط كبير موجب بين حجم تجمعات الخلايا الليمفاوية الحاملة لسى دى ٤ وسى دى ٢٨ والكرياتينين فى مرضى الذئبة الحمراء مع إلتهاب الكلية (p=0.004).

والخلاصة إن نسبة الخلايا الليمفاوية الحاملة لسى دى ٤ وسى دى ٢٨ آعلى فى مرضى الذئبة الحمراء الذين يعانون من إلتهاب الكلية عن هؤلاء الذين لا يعانون من إلتهاب فى الكلية وتظهر تغيرات ذات دلالة إحصائية فى الإرتباط مع مستوى الكرياتينين فى الدم.