Research Article

Flowcytometric Determination of T-lymphocyte expression of CD4+ and CD8+ cells in systemic lupus erythematosus

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Introduction

SLE is a systemic autoimmune condition characterised by a wide spectrum of clinical manifestations, partly related to the disease itself, but also linked to its comorbidities and drugs adverse reactions. Following the previous annual reviews, we focused on new insights in SLE clinical features, pathogenic pathways, biomarkers of specific organ involvement and therapeutic strategies. Finally concentrated on SLE aspects that could significantly influence patients' quality of life and that need to be investigated in detail through the development and validation of disease specific patient-reported outcomes. [(1)].

Rate of SLE varies between countries from 20-70/100,000[(2)]. Women are affected about 9 times more than men[(3)], begins between the ages of 15 and 45[(4)] SLE is an autoimmune disorder caused by a complex combination of genetic, epigenetic and environmental factors that lead to altered gene expression and function of several molecules which lead to abnormal T cell responses [(4)].

Aim of the work

In this study we aimed to provide more scientific insights into the flow cytometric analysis to quantify the T-lymphocyte expression of $CD4^+$ and $CD8^+$ cells in systemic lupus erythematosus patients.

Patients and methods

This study was conducted along the period from March 2018 to March 2019 on patients diagnosed as having systemic lupus erythematosus who were compared with a group of apparently healthy volunteers. The patients were selected from those who attended Rheumatology and Nephrology outpatient clinics, Minia University Hospital, Egypt.

Therefore, the current study included two groups:

Group I: It consists of 40 apparently healthy women to serve as a control group. Their ages ranged from (18-35) years with mean ± SD of (26.8 ± 5.4) years.

Group II: It comprised 40 SLE women. Their ages ranged from (16-37) years with mean ± SD of (24.7 ± 5.3) years.

The diagnosis of SLE was done according to Systemic Lupus Erythematosus International Collaborating Clinics (CLICC) 2018 and SLE sheet and systemic lupus erythematosus diseases activity index Selene modification (SLEDAI Score)

Exclusion criteria:

1- Rheumatic disease other than SLE.
2- SLE patients who had prior treatment with monoclonal antibodies or other biological drugs.
3- Malignant tumours.
4- Ongoing infections.

All patients and controls were subjected to:

1. **Careful History taking**;

Including: name, age, sex, residence, family history, consanguinity, marital status, occupation diseases, menstrual and obstetric history.

2. **Clinical Examination**;

Including: general examination, locomotors examination and neurological examination.

3. **Laboratory Investigation**:

A- Routine investigations:

- *Complete blood count (CBC).*
- Erythrocyte sedimentation rate (*ESR)*
- *Liver function tests*

- *Renal function tests*
- *Urine analysis*
- *24 hour urine proteins*
- *Rheumatoid factor(RF)*
- *C-reactive protein(C-RP)*

A- Analysis of data:

Analysis was carried out using a (PD-FACS FLOW Argon Laser U.S.A.) Flow cytometry at 515 nm.

Data processing was carried out with the XL software.

Results

Concerning the percentage of CD4 lymphocytes expression it was statistically significantly lower in SLE patients than controls (30.5 (27.3-32) vs. 35.9 (33.6-38.3) %, p<0.001). Similar trend was observed for CD8 lymphocytes expression (22.1 (16.1-25.1) % for SLE patients vs. 25.4 (21.3-29) % for controls, p=0.007).

Table 1: Results of flow cytometric analysis in the study groups

Variable		Control N=40	Cases N=40	P value
Lymphocytes CD4 expression (%)	*Median IQR*	35.9 (33.6-38.3)	30.5 (27.3-32)	***<0.001****
Lymphocytes CD8 expression (%)	*Median IQR*	25.4 (21.3-29)	22.1 (16.1-25.1)	***0.007****

Apart from a significant correlation between CD4 and CD8 lymphocyte expression (r=0.847, p<0.001), no significant correlation could be detected between either CD4, CD8 and any of the studied parameters.

Table (2): Correlation between CD4 lymphocyte expression and different studied parameters in systemic lupus erythematosus patients

Variable	CD4 lymphocyte expression (%)	
	R	P value
Age (years)	0.088	0.591
Duration of disease (years)	0.112	0.491
White blood cells ($x10^3$/µl)	-0.020	0.904
Red blood cells ($x10^3$/µl)	-0.187	0.249
Hemoglobin (gm/dl)	-0.171	0.292
Platelets ($x10^3$/µl)	-0.020	0.901
Absolute Lymphocytes count ($x10^3$/µl)	-0.175	0.280
Alanine aminotransferase (U/L)	0.140	0.389
Aspartate transaminase (U/L)	0.070	0.669
Total Bilirubin (mg/dl)	0.114	0.485
Direct Bilirubin (mg/dl)	-0.256	0.110
Albumin (g/dl)	-0.133	0.415
Urea (mg/dl)	0.143	0.377
Creatinine (mg/dl)	-0.104	0.523
First hour erythrocyte sedimentation rate (mm/h)	-0.231	0.151
Second hour erythrocyte sedimentation rate (mm/h)	-0.268	0.095
Rheumatoid factor (-ve/+ve)	-0.289	0.071
C-reactive protein (-ve/+ve)	-0.171	0.290
Proteinuria /24hour (mg)	0.065	0.688

Table (3): Correlation between CD8 lymphocyte expression and different studied parameters in systemic lupus erythematosus patients

Variable	CD8 lymphocyte expression (%)	
	r	P value
Age (years)	0.051	0.752
Duration of disease (years)	0.075	0.647
White blood cells ($x10^3$/µl)	-0.123	0.449
Red blood cells ($x10^3$/µl)	-0.298	0.062
Hemoglobin (gm/dl)	-0.161	0.320
Platelets ($x10^3$/µl)	-0.221	0.171
Absolute Lymphocytes count ($x10^3$/µl)	0.060	0.715
Alanine aminotransferase (U/L)	0.034	0.837
Aspartate transaminase (U/L)	-0.022	0.892
Total Bilirubin (mg/dl)	0.105	0.520
Direct Bilirubin (mg/dl)	-0.122	0.452
Albumin (g/dl)	-0.150	0.356
Urea (mg/dl)	0	0.999
Creatinine (mg/dl)	-0.161	0.321
First hour erythrocyte sedimentation rate (mm/h)	-0.244	0.129
Second hour erythrocyte sedimentation rate (mm/h)	-0.367	0.020
Rheumatoid factor (-ve/+ve)	-0.330	0.037
C-reactive protein (-ve/+ve)	-0.237	0.141
Proteinuria /24hour (mg)	0.053	0.746

Discussion

SLE is recognized as chronic, often severe autoimmune disease with largely unknown etiology [4].

In this study we found significantly lower levels of $CD4^+$ lymphocyte expression in patients with SLE compared to healthy volunteers ($p<0.001$). Similar trend was observed for $CD8^+$ lymphocyte expression ($p=0.007$)

Although there was no significant correlation between % of lymphocyte expression of either $CD4^+$ or $CD8^+$ with any of the studied parameter, we noticed a significant positive correlation between $CD4^+$ and $CD8^+$ ($p<0.001$). We found that the area under ROC curve of lymphocyte expression of $CD4^+$ and $CD8^+$ were 0.815 (95% CI= 0.712-0.893, $p<0.001$), at a cutoff value of < 32% and 0.675 (95% CI= 0.561-0.776, $p=0.004$) at a cutoff value of < 18.8, respectively. CD4 lymphocyte expression exhibited sensitivity, specificity, PPV, NPV, and accuracy of 77.5%, 87.5%, 86.1%, 79.5% and 82.5%, respectively, whereas those of CD8 were 32.5%, 100 %, 100%, 59.7% and 66.2%, respectively.

These results go hand with hand with those Zahran et al., [5] who studied the effects of royal jelly (RJ) supplementation on regulatory T cells in 20 SLE children received 2 g of freshly prepared RJ daily, for 12 weeks and resulted in children with SLE, before treatment there was an observed imbalance between CD4+ and CD8+ lymphocytes; this may be explained by the immune dysregulation in cases of SLE. Their results showed that patients with SLE (both before and after RJ treatment). The frequency of CD4+ T lymphocytes was significantly increased after RJ treatment versus baseline value.

This was not true for CD8+ T lymphocytes as it did not show any significant changes

with RJ treatment or any difference between the SLE children and normal control group.

Our results are in accordance with what was found by Zhang et al.,[5] who examined the levels and function of peripheral blood immunoregulatory T-cell subpopulations in SLE. They found normal percentages of CD8+ T cells in peripheral blood in all SLE patients. Interestingly, about CD4+, they found about half of the SLE patients had markedly depressed CD4+ cell levels and in turn significantly lower CD4+/ CD8+ cell ratio, whereas the remaining half of the patients had normal levels of CD4+ cells (normal CD4+/CD8+ cell ratio) [5].

References

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