T-cell markers in systemic lupus erythromatosus patients

Mostafa Draws1*, Randa M. Talaat1, Laila A. Rashed2

1Molecular diagnostics department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt
2Clinical Pathology Department, Faculty of Medicine, Cairo University

Corresponding author: Mostafa Drawy Hassen       Email: aeru1000@yahoo.com

ABSTRACT

Systemic lupus erythematosis (SLE) is an autoimmune disease characterized by the presence of multiple autoantibodies. Detecting the expression of CD antigens is supposed to be a novel diagnosis method. Therefore, the aim of current study was to investigate the importance of markers (CD 69 and CD 73) on T-cells in the diagnosis of SLE; using flowcytometric analysis. The study was carried out on healthy subjects (control) and patients with active SLE and patients with active SLE + HCV. Blood count markers were significantly reduced while serum creatinine and ANA index significantly elevated in patient groups compared to the control. CD 69 was quantified using flowcytometric technique. The first was significantly increased in both SLE group and SLE+ HCV group compared to control. A reverse pattern was obtained with CD 73 that was decreased in patient groups. Collectively, flowcytometric technique provided an accurate and reproducible tool for determination of new markers CD 69 and CD 73 on T cells. The first marker CD 69 exhibited good ability to predict the presence of SLE and the second one CD 73 exhibited excellent ability to predict the absence of SLE in patients with or without HCV.

Key words: SLE, CD, HCV

1. INTRODUCTION

An autoimmune disease is a condition in which the immune response is directed toward destruction of healthy body tissues including the heart, brain, nerves, muscles, skin, eyes, joints, lungs, kidneys, glands, the digestive tract, and blood vessels (Konforte et al., 2012). One of the most diverse of the autoimmune diseases is Systemic Lupus Erythematosus (SLE), characterised by a wide range of clinical features and the production of multiple autoantibodies (Bertolaccini et al., 2008). Most of these antibodies are directed to double stranded DNA (ds-DNA), nuclear antigens, ribonucleoproteins and cell surface antigens. The incidence of SLE varies from 2 to 7.6 cases per 100,000 individuals per year (Bertolaccini et al., 2008).

Therefore, the laboratory hallmark of the disease is the presence of antinuclear antibodies (ANA), appearing in more than 95% of individuals with lupus (Bertolaccini et al., 2008). While the history and physical examination are most important in assessing disease activity, laboratory tests are helpful in organ systems (hematologic, renal) that cannot be assessed clinically. It is worthy noted that ANA alone is not a satisfactory marker for assessing disease prognosis (Lam and Petri, 2005). The cluster of differentiation antigens (CD antigens) are membrane proteins mainly expressed on leukocytes. A small number are also expressed on endothelial cells, erythrocytes, and stem cells. Recently, CD antigens are commonly used as cell markers,
allowing cells to be defined based on what molecules are present on their surface. Therefore, detecting the expression of CD antigens is supposed to be developed as novel diagnosis methods for some diseases (Zola et al., 2005). Interestingly, abnormal expression of key molecules and defective function of T lymphocytes might play a significant role in the pathogenesis of SLE (Nambiar et al., 2004). T cells from patients with SLE reveal atypical responses to stimuli that contribute to the persistence of autoreactive clones and the lack of productive immune responses. Thus, defects in the mechanisms that regulate the activation and proliferation of T cells could be accountable for the numerous changes detected in these patients (Kyttaris and Tsokos, 2004). CD69 is one of the earliest specific antigens acquired during lymphoid activation. It is widely expressed in a variety of cells of the hematopoietic lineage, including neutrophils, monocytes, T cells, B cells, natural killer (NK) cells and platelets (Natarajan et al., 2000). Notably, activation of T cells induces the expression of CD69 at the cell surface (Natarajan et al., 2000).

It is worth mentioning that CD73 plays a critical role in host defense (Yegutkin, 2008). It is involved in lymphocyte adhesion to endothelium and restriction of lymphocyte migration into draining lymph nodes (Takedachi et al., 2008), costimulation of T cell activation, and control of B cell–follicular dendritic cell interaction (Airas and Jalkanen, 1996).

Therefore, the aim of current study was to investigate the importance of 2 new markers (CD69 and CD73) on T-cells in the diagnosis of SLE using flowcytometry as a new molecular technique.

2. SUBJECTS AND METHODS

This study was carried out on 98 subjects divided into 3 groups whose ages ranged from (30-56) years. Briefly, group 1 served as a control and consisted of 30 healthy subjects. Group 2 consisted of 42 SLE patients while group 3 contained 26 SLE patients in addition to HCV. Diagnosis was made according to the “American College of Rheumatology” revised criteria for the classification of SLE. All patients were diagnosed and received regular treatment for SLE in the “Inpatient clinic of Rheumatology and Rehabilitation department”, Faculty of Medicine, Cairo University hospitals.

Blood samples were obtained by venipuncture from all groups. Each blood specimen was divided into 4 sterile tubes as follows:
1. EDTA vacutainer tube for measuring of complete blood count (CBC) in whole blood.
2. Sodium citrate vacutainer tube for measuring of erythrocyte sedimentation rate (ESR) in whole blood.
3. Plain vacutainer tube for measuring of antinuclear antibody (ANA) and creatinine in sera.

2.1. Analysis of Complete Blood Count:

Parameters of CBC were measured with hematology analyzer (XE-2100, System Corp., Kobe, Japan). Measured CBC parameters included HGB (gm/dL), leukocytes (cell/µL) and thrombocytes (cell/µL).
2.2. Determination of Erythrocyte Sedimentation Rate:
This test was carried out using the classical Westergren method according to Thomas et al. (1993). The ESR was calculated by transformation to the Westergren tube scale (200 mm height) using the following equation:
\[ \text{ESR} = (1 - \text{percentage of sedimentation}) \times 200. \]
The unit of erythrocyte sedimentation by this method was millimeter per hour (mm/hr) and readings were recorded at 1 hr.

2.3. Assessment of Antinuclear antibody using ELISA:
The ELISA kit for ANA was obtained from Alpha Diagnostic International (Texas, USA). The technique depends on semi-quantitative indirect solid phase enzyme immunoassay, according to the manufacturer instructions. Briefly, the kit had a specific microplate pre-coated with monoclonal antibody against ANA. After adding the standards and samples, the analyte was sandwiched by the immobilized antibody and HRP-conjugated anti-human immunoglobulin (conjugate). All unbound material was then washed away. Thereafter, a chromogenic substrate solution containing TMB was added and enzymatically oxidized by HRP. The oxidized product of TMB had a blue color that changed yellow after adding the stop solution (sulphoric acid). The optical density was measured at 450 nm using a microplate reader (ChroMate-4300, FL, USA). The intensity of the color was directly proportional to the sample concentration (Reichlin, 1980).

2.4. Assay of serum creatinine:
This parameter was measured by Spectrum Diagnostics creatinine reagents kit (Egyptian Company for Biotechnology, Cairo, Egypt). The assay depends on the reaction between creatinine and picric acid under alkaline condition to form a yellow-red complex. The absorbance was then measured at 492 nm using Shimadzu UV-VIS spectrophotometer 1650 (Tokyo, Japan). The color is directly proportional to creatinine concentration in the sample (Giorgio, 1974).

2.5. Isolation of human peripheral blood mononuclear cells (PBMCs):
PBMCs were isolated from whole blood obtained from healthy donors of whom informed consent was obtained that their donated blood might be used for scientific purposes. Blood specimen was diluted with phosphate buffered saline (PBS) addition of Ficoll reagent (Sigma Chemical Co., St. Louis, MO) on diluted blood slowly. Separation of PBMCs was performed using density centrifugation (Lymphocyte Separation Medium, Lonza, Basel, Switzerland) according to the manufacturers’ instructions. Aspiration of cloudy layer (containing of PBMC) in between upper Ficoll reagent layer and lower blood content. After isolation, cells were washed three times in phosphate buffered saline (PBS) containing 1 µM EDTA followed by centrifugation then discarding the supernatant. One mL of cold absolute ethanol was added on precipitated cells before storage.

2.6. Isolation of T-cells from PBMCs:
T-cells were isolated from PBMCs by using of Dynabeads® Untouched™ Human T Cells Kit (Invitrogen® life technologies, CA, USA). The technique depends on depleting all cell types except T cells, according to the manufacturer instructions. In brief, the kit had a mixture of mouse IgG antibodies against the non-T cells added to the starting sample. Depletion Dynabeads® were added and bind to the antibody-labeled cells during a short incubation. The bead-bound cells were subsequently separated on a magnet (Dynal MPC™-6, Invitrogen® life technologies, CA, USA) and discarded. The supernatant contained the untouched human T cells.
2.7. Fluorescence-activated cell sorting (FACS) staining and analysis:

In all cases, sample preparation and flow cytometric (FCM) data acquisition were performed within the first 24 hr from collection. The antibodies clones were carefully selected on the basis of their reactivity patterns. The FCM analysis was constructed so that a double fluorochrome conjugated monoclonal antibody combination was established as CD69 and CD73 FITC units (Future. Innovation. Technology. Creativity).

The final cell suspension in FACS buffer was taken in 2 mL of FACS tube, then washed twice with 2mL of PBS/BSA, centrifuged at 2000 rpm for 8 minute at 4°C and the pellets were re-suspended in 2mL PBS. Subsequently, the cells were ready for staining by incubation with Mouse Anti-Human Cd69 1st antibody with label (FITC) (BD pharmina, BD biosciences, Heidelberg, Germany) for 15 min on ice, followed by washing of cells using of PBS/BSA, then centrifugation at 2000 rpm for 5 min. Re-suspended pellets were incubated another time with Mouse Anti-Human Cd73 2nd antibody (BD pharmina, BD biosciences, Heidelberg, Germany) with other Fluorochrome for 20 min at room temperature in dark. Thereafter, cells were washed using PBS/BSA, then centrifuged at 2000 rpm for 5 min. Pellets were re-suspended in 4% parformahlehyde to acquire on FCM.

All data of FCM analysis were acquired by BD Accuri™ C6 Flow Cytometer (BD biosciences, USA) supplied with BD Accurri C6 Software for data processing. Gating of cells was initially set according to light scatter on a forward scatter (FS) and side scatter (SC) to exclude cellular debris and to identify the T-lymphocyte population. Data obtained was recorded in the form of percent of cells expressing the markers and the mean intensity of the expression termed mean fluorescence intensity (MFI).

2.8. Statistical analysis

Statistical analysis was performed using SPSS statistical package version 22. Qualitative data were presented as frequency and percentages. Quantitative variables were presented as Mean± Standard deviation (SD) and range or median and inter-quartile range (IQR) (in case of violation of normality assumption). Normality assumption was tested using Shapiro-wilk test and Q-Q plot. One way Analysis of variance (ANOVA) test and the Kruskal-wallis test (in case of violation of normality assumption) were used to compare the quantitative variables. Chi squared test was used to compare qualitative variables. Spearman correlation was used to test correlations between two quantitative variables. Receiver operating curve (ROC) was used to compare the ability of CD69 and CD73 to predict the presence of systemic lupus erythematosus. Variables that showed statistical significance at univariate analysis were further chosen for multiple linear regression. Backward selection method was adopted to choose the most significant variables that should be included in the final model. Level of Significant difference was considered at a two-sided p-value < 0.05.

3. Results:

3.1. Demographic data:

The demographic data of the present study are summarized in Tables (1, 2 and 3). The study was carried out on 30 healthy subjects who served as control, 42 patients with active SLE and 26 patients with active SLE + HCV, as indicated in Table 1. Notably, all subjects and patients were generally of middle-age group with average
age of about 44 years; however, the mean age of SLE + HCV patients was significantly higher than the control, as shown in Table 2. As for the sex distribution, all groups were matched. Each group obviously consisted of males (one-third) and females (two-thirds), as shown in Table 3.

Table (1): Demographic characters of the study population

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>44.55</td>
<td>5.71</td>
<td>31 - 56</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>31</td>
<td>31.6</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>68.4</td>
<td></td>
</tr>
</tbody>
</table>

Table (2) Comparing age in the three study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLE</th>
<th>SLE + HCV</th>
<th>ANOVA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>42.1 ± 5.006</td>
<td>44.9 ± 6.1</td>
<td>46.81* ± 4.86</td>
<td>5.39</td>
<td>0.007</td>
</tr>
</tbody>
</table>

After performing post hoc bonferroni adjustment the statistical significance was found to be between Control group and SLE + HCV group (p-value = 0.005).

Table (3) Comparing sex distribution in the three study groups

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLE</th>
<th>SLE + HCV</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 (32.3)</td>
<td>13 (41.9)</td>
<td>8 (25.8)</td>
<td>0.058</td>
<td>0.97</td>
</tr>
<tr>
<td>Female</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20 (29.9)</td>
<td>29 (43.3)</td>
<td>18 (26.9)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Groups are matched regarding sex distribution.
3.2. Assessment of blood count, serum creatinine and ANA:

In order to assess the effect of SLE and HCV on blood count: HGB concentration, leucocytic and thrombocytic counts were measured. As shown in Table 4, HGB concentration was significantly reduced in both SLE group and SLE+ HCV group by about 12.5% and 12.8% from the control, respectively. In addition, leucocytic count was significantly decreased in both SLE and SLE+ HCV groups by 14.5% and 20%, respectively, compared to the control. A similar pattern of observations was detected with thrombocytic count. On contrary, SLE group and SLE+ HCV group have shown significant more than two-fold increase of serum creatinine, compared to the control. This effect was highly pronounced with ANA level that was significantly increased by 18 folds in SLE group and 13 folds in SLE+ HCV group, compared to the control.

<table>
<thead>
<tr>
<th>Table (4): Comparing blood cell count, kidney functions, ANA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HGB (g/dL)</strong></td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Leucocytes (cell/µL)</strong></td>
</tr>
<tr>
<td><strong>Thrombocytes (cell/µL)</strong></td>
</tr>
<tr>
<td><strong>s.creatinine (mg/dL)</strong></td>
</tr>
<tr>
<td><strong>ANA index</strong></td>
</tr>
</tbody>
</table>

Data are presented as Mean ± SD
Statistical analyses were carried out using One-way ANOVA followed by Bonferroni post-hoc test
*significantly different from the corresponding control at p-value <0.001.
*Welch adjustment was performed due to unequal variances.

3.3. Assessment of ESR, CD69 and CD73:

The effect of SLE and HCV on ESR, and the leukocytic markers (CD 69 & CD 73) were further assessed. As indicated in Table 5, ESR was significantly elevated in both SLE group and SLE+ HCV group by about 8 folds from the control. A similar pattern of activity was detected with CD 69 that was significantly increased in both SLE group and SLE+ HCV group by about 2 folds & 4 folds from the control, respectively, as shown in table 5 and Figure 1A. In contrast, CD 73 was significantly decreased by about 31% & 36% in both disease groups (SLE & SLE+ HCV), compared to the control group, as shown in table 5 and Figure 1B.
Table (5): Comparing ESR, CD69, CD73 (not normally distributed parameters)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLE</th>
<th>SLE + HCV</th>
<th>$X^2$ (kruskal-wallis)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESR (1st hour)</strong></td>
<td>6.00 (3.00)</td>
<td>51.5$#$ (14.5)</td>
<td>50.5$#$ (12.25)</td>
<td>62.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>CD69 (%)</strong></td>
<td>4.4 (2.28)</td>
<td>9.25$#$ (7.23)</td>
<td>16.9$#$ (13.2)</td>
<td>52.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>CD73 (%)</strong></td>
<td>49.0 (11.08)</td>
<td>33.6$#$ (13.75)</td>
<td>31.1$#$ (11.57)</td>
<td>41.78</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as median (IQR)
Statistical analyses were carried out using Kruskal-Wallis followed by Bonferroni post-hoc test
$\#$ significantly different from the corresponding control at $p$-value <0.001.

**Figure 1**: Assessment of CD69 (Panel A) and CD73 (Panel B) in healthy subjects, patients with SLE and patients with SLE + HCV
Data are presented as median (IQR)
Statistical analyses were carried out using Kruskal-Wallis followed by Bonferroni post-hoc test
$\#$ significantly different from the corresponding control at $p$-value <0.001.
3.4. CD 69 & CD 73 as predictors for SLE:

As shown in Table 6 and Figure 2A, the receiver operating curve (ROC) of CD 69 exhibited good ability to predict the presence of SLE with an AUC value of 0.883 (95% CI, 0.814 – 0.952). The optimal cut-off point was found at the value (6.55) where sensitivity = 82.4% and specificity= 90%. Similarly, As indicated in Table 6 and Figure 2B, the ROC of CD 73 exhibited excellent ability to predict the absence of SLE with an AUC value of 0.907 (95% CI, 0.849 – 0.965). The highest sensitivity and specificity were found at the cut-off value (40.45) where sensitivity = 82.4% and specificity= 86.7%.

Table (6): Area under Receiver operating curve (ROC) evaluating CD69 & CD73 as predictors of SLE

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>Std. Error</th>
<th>Significance</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD69</td>
<td>0.883</td>
<td>0.035</td>
<td>&lt; 0.001</td>
<td>0.814 – 0.952</td>
</tr>
<tr>
<td>CD73</td>
<td>0.907</td>
<td>0.03</td>
<td>&lt; 0.001</td>
<td>0.849 – 0.965</td>
</tr>
</tbody>
</table>

AUC: Area Under the Curve, Std.error: Standard error.
3.5. Multiple linear regressions between quantitative variables and CD69 & CD73:

As shown in Table 7, patients with SLE showed on average 1.74-fold increase (95% CI: 0.631 – 4.797) in CD69 level than control group after adjusting for the other variables. However, this association did not reach the statistical significance level (p-value = 0.28). On the other hand, patients with SLE+ HCV showed 4.831 fold increase in CD69 level than control group after adjusting for other variables. This association between remained statistically significant after adjusting for other variables (p-value = 0.002).

Table (7): multiple linear regression using backward selection method for CD69*.

<table>
<thead>
<tr>
<th>Final variables in the model</th>
<th>b***</th>
<th>Std. Error**</th>
<th>Significance</th>
<th>95% Confidence Interval***</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>1.740</td>
<td>0.511</td>
<td>0.28</td>
<td>0.631 – 4.797</td>
</tr>
<tr>
<td>SLE+ HCV</td>
<td>4.831</td>
<td>0.489</td>
<td>0.002</td>
<td>1.827 – 12.768</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>0.921</td>
<td>0.040</td>
<td>0.042</td>
<td>0.851 – 0.997</td>
</tr>
<tr>
<td>thrombocytes</td>
<td>0.996</td>
<td>0.003</td>
<td>0.182</td>
<td>0.989 – 1.002</td>
</tr>
<tr>
<td>ESR</td>
<td>0.968</td>
<td>0.007</td>
<td>&lt;0.001</td>
<td>0.953 – 0.982</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>1.367</td>
<td>0.213</td>
<td>0.14</td>
<td>0.895 – 2.087</td>
</tr>
<tr>
<td>ANA</td>
<td>1.134</td>
<td>0.033</td>
<td>&lt;0.001</td>
<td>1.062 – 1.210</td>
</tr>
</tbody>
</table>

* CD69 was transformed to log scale to comply with normality assumption.
** Standard error was calculated in the log scale.
*** b coefficient and 95% CI were exponentiated to the normal scale.

On average, patients with SLE showed 16.528 lower CD73 level than control group after adjusting for the other variables, as shown in Table 8. Also, patients with SLE+ HCV showed 17.526 lower CD73 level than control group after adjusting for other variables. The association between CD73 and the studied groups remained statistically significant after adjusting for other variables (p-value = 0.005 & 0.005, respectively).
Table (8): multiple linear regression using backward selection method for CD73

<table>
<thead>
<tr>
<th>Final variables in the model</th>
<th>b</th>
<th>Std. Error</th>
<th>Significance</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>-16.528</td>
<td>5.752</td>
<td>0.005</td>
<td>-27.954 -5.102</td>
</tr>
<tr>
<td>SLE+ HCV</td>
<td>-17.526</td>
<td>6.019</td>
<td>0.005</td>
<td>-29.482 -5.570</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.526</td>
<td>0.182</td>
<td>0.005</td>
<td>-0.887 -0.165</td>
</tr>
<tr>
<td>HGB (gm/dl)</td>
<td>4.088</td>
<td>1.603</td>
<td>0.012</td>
<td>0.903 7.272</td>
</tr>
<tr>
<td>Thrombocytes</td>
<td>-0.265</td>
<td>0.904</td>
<td>0.006</td>
<td>-0.452 -0.077</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>-6.825</td>
<td>3.529</td>
<td>0.056</td>
<td>-13.836 0.185</td>
</tr>
</tbody>
</table>

4. Discussion:

Systemic lupus Erythromatosis is characterized by the presence of multiple autoantibodies (Bertolaccini et al., 2008). Although the presence of ANA has been considered as the laboratory hallmark of SLE, ANA alone is not a satisfactory marker for assessing disease prognosis (Lam and Petri, 2005). In addition, detecting the expression of CD antigens is supposed to be developed as novel diagnosis methods (Zola et al., 2005). Therefore, the aim of current study was to investigate the importance of 2 new markers (CD69 and CD73) on T-cells in the diagnosis of SLE; using flowcytometry as a new molecular technique.

The current study was carried out on age and sex-matched groups of healthy subjects and SLE patients with and without HCV in order to minimize inter-individual variability. It is worth mentioning that viral infections in SLE have been suspected to play a pathogenic role on development, trigger and flare of disease. This might be explained by activation of immune system and antibodies production during acute viral infections. On the other hand, acute viral infections are frequently reported at disease presentation, confusing and favoring misinterpretation of clinical signs and deferral of adequate treatment (Ramos-Casals et al, 2008). Therefore, a group of SLE with HCV infections was included in the present study.

Furthermore, the effect of SLE on hematological parameters was confirmed by measuring HGB, leucocytic & thrombocytic counts and ESR. HGB and cell counts were significantly decreased while ESR was markedly elevated, from the healthy control subjects. A growing body of evidence supports these findings. Ben-Menachem (2010) stated the wide recognition of hematological disorders in SLE that are characterized by lymphopenia, anemia and thrombocytopenia. Moreover, Salchi-Abari (2015) and Bertsias et al., (2015) have shown that cytopenias (Leucopenia, thrombocytopenia and hemolytic anemia) are useful in establishing the diagnosis of SLE.

Regarding the effect of SLE on renal function, serum creatinine was assessed in patients and found to be significantly increased compared to the control subjects. This finding confirmed the deleterious effect of SLE on renal function leading to lupus nephritis. In harmony with the current results, many studies have shown that decline in renal functions is a strong evidence of lupus nephritis (Cervera et al., 2003; Bertsias et al., 2008; Bertsias et al., 2015). It is well-known that ANA detection is the current laboratory hallmark of SLE, appearing in more than 95% of individuals with lupus (Bertolaccini et al., 2008). In the current study, there was a marked increase of ANA in all SLE patients with or without HCV.
Taken together with hematological and renal changes, it was confirmed that all patients in the current study fulfilled the most important criteria for SLE diagnosis. Flow cytometry was discovered in 1980s as a technique for analyzing the expression of cell surface and intracellular molecules (Bohn, 1980; Norman 1980). It is also important for characterizing and defining different cell types in heterogeneous cell populations, assessing the purity of isolated subpopulations. Further it is beneficial in analyzing cell size & volume and allows simultaneous multi parameter analysis of single cells (Dethlefsen et al., 1980). Mechanistically, it is predominantly used to measure fluorescence intensity produced by fluorescent labeled antibodies detecting proteins or ligands that bind to specific cell associated molecules (Fullwyler, 1980). In the present study, the expression of CD parameters “CD69 and CD73” was investigated using flowcytometric analysis for its accurate and reproducible results. There was a significant increase of CD69% in SLE patients with or without HCV. This increased CD69 expression on T cells might be due to an intrinsic T cell defect or abnormal humoral factors in SLE patients. Previous In-vitro studies had demonstrated the role of IL-10 to CD69 expression. In this regard, adding anti-IL-10 antibodies to the peripheral blood mononuclear cell culture promotes CD69 expression in cell cultures of SLE patients, compared to control subjects. The increase was detected in the cells with higher basal CD69 expression, suggesting the inhibitory role IL-10 that compensates for T-cell hyperactivity (Wang et al., 2005). In a related study involving T cell stimulation assays, T cells from patients with active SLE were found to have an intrinsic defect that impairs their activation process, probably involving an alteration in the activation of protein kinase C (Crispin et al., 1998). It is worthy noted that CD69 expression has been studied in rheumatoid arthritis as another autoimmune disease. It was shown that CD69 antigen expression was significantly elevated on peripheral blood and synovial fluid neutrophils from rheumatoid patients (Atzeni et al., 2004). In other studies, CD69 antigen was over-expressed in T cells obtained from synovial fluid of patients with rheumatoid arthritis and juvenile rheumatoid arthritis, compared to peripheral blood T cells from the same patients or to normal controls (Fernandez-Gutierrez et al., 1995; Minami et al., 2006). Therefore, the up-regulation of CD69 might be an indicator of the high proportion of activated lymphocytes that accumulate in the affected joints rather than the result of an intrinsic cell defect as in SLE.

The other predictor “CD73” is a 70-kDa protein with ecto 59-nucleotidase enzyme activity that catalyses the generation of adenosine from AMP by dephosphorylation (Yegutkin, 2008). Various studies have shown the role of CD73 in the generation of extracellular adenosine in several physiologically-relevant experimental models (Hasegawa et al., 2008; Peng et al., 2008; Reutershan et al., 2009). However, CD73 has additional functions in host defense (Yegutkin, 2008), lymphocyte adhesion to endothelium and restriction of lymphocyte migration into draining lymph nodes (Takedachi et al., 2008), stimulation of T cell activation with the control of B cell–follicular dendritic cell (DC) interaction (Airas and Jalkanen, 1996). Notably, CD73 is particularly highly expressed in regulatory T (Treg) cells, which can suppress effector T cells by converting AMP to adenosine (Mandapathil et al., 2010; Romio et al., 2011). The current data demonstrated that CD73 was significantly decreased in SLE patients and SLE + HCV patients. This was in harmony with the previous study of Li et al. (2010); in which CD73 expression in T regulatory cells was decreased in active SLE patients as compared to healthy controls and inactive SLE patients. On contrast, it is worth mentioning that enhancing CD73 signaling was proven to be a useful tool for suppression of T regulatory cells and/or decreasing their number, which might be beneficial in systemic autoimmune diseases (Miyara et al., 2011).
5. CONCLUSION

Flowcytometric technique provides an accurate and reproducible tool for determination of 2 new markers CD69 and CD73 on T cells. The first marker CD69 exhibited good ability to predict the presence of SLE and the second one CD73 exhibited excellent ability to predict the absence of SLE in patients with or without HCV.

6. REFERENCES


3.2. Assessment of blood count, serum creatinine and ANA:

In order to assess the effect of SLE and HCV on blood count: HGB concentration, leucocytic and thrombocytic counts were measured. As shown in Table 4, HGB concentration was significantly reduced in both SLE group and SLE+HCV group by about 12.5% and 12.8% from the control, respectively. In addition, leucocytic count was significantly decreased in both SLE