EXPRESSION OF CD69 ON PLATELETS OF PATIENTS WITH Systemic Lupus Erythematosus AND Cardiovascular Disease

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ABSTRACT

Introduction: Systemic lupus erythematosus is an autoimmune disease characterized by inflammation of many different systems. Epidemiological studies showed an increase of cardio and cerebrovascular events in patients suffering from systemic autoimmune diseases, and autoptic investigations pointed out that an accelerated atherosclerotic process is largely responsible for such manifestations. Both symptomatic as MI (myocardial infarction) and asymptomatic as atherosclerotic (carotid plaque) diseases are more prevalent in SLE patients than in the general population. There were positive correlations between CD69 (one of type I IFN-regulated genes in platelets) level and cardiovascular complications in these patients. Objective: To determine the level of CD69 and C3 in patients with systemic lupus erythematosus and to find the relationship between CD69 and C3 levels and cardiovascular complications in these patients. Methods: Twenty systemic lupus erythematosus patients were included in this study were selected from internal medicine in and out-patient clinics of Al-Zahraa university hospital, ten patients suffering of SLE with no cardio vascular disease (Group 1) and ten patients suffering of SLE with cardio vascular disease (Group 2). Ten healthy individuals of matched age and sex were selected as control group. All systemic lupus patients were subjected to full history taking, clinical examination, ECG, complete blood picture, liver function, renal function, ANA, dDNA, serum complement 3, and CD69 on platelets were done. Results: The expression of CD69 on platelets was significantly higher in patient of SLE with cardiovascular complication group than control group (p=0.0071), and there was a significant increases in the serum level of C3 of SLE with cardiovascular complication group than in patient of SLE without cardiovascular complication group (p=0.000005). Also we found that the serum level of C3 lower in patient of SLE group with and without cardiovascular complication than control group (p=0.009) (0.00009). Conclusion: These finding suggest that Platelets with CD69 expression seem to more activated and may contribute to development of vascular complication in patients of SLE so CD69 expression on platelets may serve as predictive marker of CVD complication in patients with SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by inflammation in many different organ systems such as skin, joints, kidney, nervous system, serosal membranes and blood elements. B-cell abnormalities, autoantibodies, complement activation and an ongoing type I interferon (IFN) production are all of importance in the pathogenesis of SLE(1,2). Type I interferon (IFN) gene signature in patients with SLE, and the IFN-regulated proteins PRKRA, IFITM1 and CD69 were found to be up-regulated in platelets from SLE patient(3).

Epidemiological studies showed an increase of cardio and cerebrovascular events in patients suffering from systemic autoimmune diseases, and autoptic investigations pointed out that an accelerated atherosclerotic process is largely responsible for such manifestations. These observations support a possible role of autoimmunity in the genesis of atherosclerosis that may have clinical or subclinical features(3).

Both preclinical (carotid plaque) and clinical (myocardial infarction) atherosclerotic diseases are more prevalent in SLE patients than in the general population. Clinically atherothrombotic events, such as myocardial infarction (MI), have been recognized as risk factors for mortality, there may be a bimodal distribution of mortality risk factors in lupus, an early peak in mortality is caused by disease activity and severity itself, as well as infections, while a late peak is related to coronary artery disease (CAD), CAD is described with a prevalence ranging from 6 to 10%, and, in SLE patients, the risk of developing any CAD is 4–8 times higher than in control. In young women with SLE, the risk of MI is increased 50-fold. In various cohort studies, MI was the cause of death in 3–30% of SLE patients(4).
The excess risk of cardiovascular disease (CVD) associated with inflammatory rheumatic diseases has long been recognized. Patients with established rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) have higher mortality compared with the general population. Over 50% of premature deaths in RA are attributable to CVD. Excess mortality in SLE follows a bimodal pattern, with the early peak predominantly a consequence of active lupus or its complications, and the later peak largely attributable to atherosclerosis. Patients with RA or SLE are also at increased risk of nonfatal ischemic heart disease. The management and outcome of myocardial infarction and congestive heart failure in patients with RA or SLE differs from that in the general population (5).

Immune dysregulation characteristic of lupus appears to play the dominant role in atherogenesis. While both SLE-specific and non-specific mechanisms have been proposed to play a prominent role in the induction of premature vascular damage in this disease (6), platelet activation have possible role in pathogenesis of CVD, SLE increased platelet expression of p-selectin and phosphatidylserin (PS) has been demonstrated, furthermore, platelet-monocyte complexes which could accelerate the production of tissue factor are found in SLE (7). Markers of sustained platelet activation such as extracellular phosphorxlation of plasma proteins fibrinogen and C3 has been found in SLE patients and may be associated with venous thrombosis (8).

CD69 is asignal transducing disulfide linked homo-dimer functionally expressed on platelets, CD3 bright thymocytes and activated lymphocytes, CD69 involved in lymphocyte proliferation and functions as asignal transmitting receptor in lymphocytes natural killer (NK) cells and platelets (9).

Upregulation of CD69 expression by IFN has been described in neutrophils and megacaryocytes (10). CD69 is a marker of activation of inflammatory cells and it is suspected to participated in pathology of vasculitis and possibly in platelet aggregation (11). Complement 3, often simply called C3, is a protein of the immune system. It plays a central role in the complement system and contributes to innate immunity. In humans it is encoded on chromosome 19 by a gene called C3 (12).

C3 plays a central role in the activation of complement system. Its activation is required for both classical and alternative complement activation pathways. People with C3 deficiency are susceptible to bacterial infection.

One form of C3-convertase, also known as C4b2a, is formed by a heterodimer of activated forms of C4 and C2. It catalyzes the proteolytic cleavage of C3 into C3a and C3b, generated during activation through the classical pathway as well as the mannan-binding lectin pathway. C3a is an anaphylotoxin abinding lectin pathway. C3a is an anaphylotoxin and the precusor of some cytokines such as ASP, and C3b serves as an opsonizing agent (13).

Observation of low complement concentrations and also of activation of the complement system are characteristic findings in active SLE and have led to the practice of using measurement of complement for the diagnosis, classification and monitoring of disease course in SLE.

In the 1982 set of ACR criteria for SLE, complement components were not included. However, low levels of CH50, C3 and C4 were tested when the criteria were developed. The sensitivities and specificities for the tested components were 70 and 70%, respectively, for CH50, 64 and 91% for C3 and 64 and 65% for C4. In further analysis by recursive partitioning on the same data set, the combined sensitivity and specificity for CH50, C3 and C4 were 74 and 88%. In a more recent study, low levels of C1q were found to have a high specificity (96%) but the sensitivity was low (20%) (14).

Aim of the work:

To determine the level of C3 and CD69 in patients with systemic lupus erythematosus and to find the relationship between C3 and CD69 levels and cardio vascular complication in these patients.

PATIENTS & METHODS

The present study was conducted on 30 subjects: twenty patients with SLE (all patients were diagnosed according to criteria of American college for Rheumatology method classification criteria for SLE), and Ten healthy individuals of matched age and sex were selected as control group. The patients were selected from internal medicine department in and out-patient clinics of Al-Zahraa university hospital from January 2011-july 2011 after informed consent. The patients group were classified into two group; Group 1: ten patients suffering of SLE with no cardio vascular disease one of them was male and 9 were females, their age ranged from 17 to
33 years, patients in this group proved to have no cardiovascular disease

**Group 2**: ten patients suffering of SLE with cardiovascular disease 2 of them were males and 8 were females, their age ranged between 26 and 40 years, patients in this group proved to have cardiovascular disease

**All groups were subjected to**

Full history taking, and full clinical examination, ECG, and Eechocardiography were done.

The following investigation were done to patients and control:

1. Blood sample were obtained in tubes, one part was put in EDTA tube for complete blood count & CD69 on platelets and the other part was put in plain tube for serum separation by centrifugation, part of the serum were used for estimation of ANA, and DNA and the other were used for estimation of serum complement 3, liver function, renal function tests. also urine analysis were done.
2. CBC was done using automated cell counter (sismix .......)
3. CD69 detected by flow cytometry model Beckman coulter machine. Kits supplied by (Beckman) (catalog V B.P177-13276 Maseillecedex France). Fifty ul of diluted blood by phosphate buffer saline (PBs) was added to 5ul of the mono clonal antibody (antihuman CD69 FITC (conjugated). The tubes vortexed and then incubated in the dark at room temperature for 15 min-1.5ml (NH4CL buffered with KHCO3 at PH7.2) was added and then vortexed, the sample was then ready for flow cytometer processing.
4. ANA and DNA detect by indirect immunofluorising technique, kits supplied from (ORGENTEC,CAT NO870-LOT870131)

**Statistical analysis**

Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16.

Parametric data was expressed as mean ± SD, and non parametric data was expressed as number and percentage of the total.

The mean and standard deviation (SD) were calculated as follows:

\[
\text{Mean (X)} = \frac{\sum (X_i)}{n}
\]

\[
\text{SD} = \sqrt{\frac{\sum (X - X_i)^2}{n - 1}}
\]

Where, \( \Sigma \): Sum.
\( X_i \): each value in the series.
\( n \): number of values in the series.

Comparing the mean ± SD of 2 groups was done using the paired t test

\[
t (df) = \frac{X - Y}{SD_p \sqrt{1/nX + 1/nY}}
\]

Where, \( t (df) \): value at the degrees of freedom.
\( df \): degrees of freedom.
\( X \): mean of sample X.
\( Y \): mean of sample Y.
\( nX \): number of sample X.
\( nY \): number of sample Y.
\( SD_p \): pooled SD (SD of both samples).

Determining the extent that a single observed series of proportions differs from a theoretical or expected distribution was done using the Chi square test

\[
X^2 (df) = \frac{\sum (O_i - E_i)^2}{E_i}
\]

Where, \( X^2 (df) \): value at the degrees of freedom.
\( df \): degrees of freedom.
\( O_i \): Observed frequency.
\( E_i \): expected frequency.

P value ≤ 0.05 is considered significant

P value ≤ 0.01 is considered highly sig P value > 0.05 is considered non-significant

**RESULTS**

The clinical and demographic data of patients groups
Table (1): Clinical and demographic data of patients groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>17-33 years</td>
<td>26-40 years</td>
</tr>
<tr>
<td>SEX</td>
<td>one male and 9 females</td>
<td>2 males and 8 females</td>
</tr>
<tr>
<td>Malar rash</td>
<td>6 patients</td>
<td>3 patients</td>
</tr>
<tr>
<td>Oral ulcers</td>
<td>3 patients</td>
<td>one patients</td>
</tr>
<tr>
<td>Photosensitivity</td>
<td>5 patients</td>
<td>4 patients</td>
</tr>
<tr>
<td>Arthritis</td>
<td>6 patients</td>
<td>5 patients</td>
</tr>
<tr>
<td>Serositis</td>
<td>2 patients</td>
<td>One patients</td>
</tr>
<tr>
<td>Renal disease</td>
<td>4 patients</td>
<td>3 patients</td>
</tr>
<tr>
<td>Neurological disease</td>
<td>-ve</td>
<td>One patient</td>
</tr>
<tr>
<td>Venous disease</td>
<td>-ve</td>
<td>4 patients with DVT</td>
</tr>
<tr>
<td>Arterial disease</td>
<td>-ve</td>
<td>2 patients with PVD</td>
</tr>
<tr>
<td>MI</td>
<td>-ve</td>
<td>6 patients</td>
</tr>
<tr>
<td>HHD</td>
<td>-ve</td>
<td>4 patients</td>
</tr>
<tr>
<td>ANA</td>
<td>+ve in all patients</td>
<td>+ve in all patients</td>
</tr>
<tr>
<td>Anti DNA</td>
<td>+ve in all patients</td>
<td>+ve in all patients</td>
</tr>
<tr>
<td>CBC</td>
<td>Aneamia8 patients</td>
<td>Aneamia6 patients and 3 patients thrompocytopenia</td>
</tr>
</tbody>
</table>

Table (2): Comparison between patients (group 1&2) and control regard CD69

<table>
<thead>
<tr>
<th>CD69</th>
<th>Control</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>30.15</td>
<td>39.40</td>
<td>51.10</td>
</tr>
<tr>
<td>SD</td>
<td>14.86</td>
<td>17.49</td>
<td>16.03</td>
</tr>
<tr>
<td>Min</td>
<td>8.3</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Max</td>
<td>46</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>T value</td>
<td>-1.274263046</td>
<td>-3.030542007</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.218776745</td>
<td>0.007197701</td>
<td></td>
</tr>
</tbody>
</table>

The expression of CD69 on platelets was significantly higher in group 2 than control group (p<0.0071) (table-2), and no significant difference in its level in group 1 (p=0.21) (table-2) when compared to control group.

Table (3): Comparison between patients (group 1&2) regard CD69

<table>
<thead>
<tr>
<th>CD69</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
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<td>16.03</td>
</tr>
<tr>
<td>Min</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Max</td>
<td>64</td>
<td>70</td>
</tr>
<tr>
<td>T value</td>
<td>-1.55926</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.136343</td>
<td></td>
</tr>
</tbody>
</table>

![Fig. (1): Comparison between patients and control regard CD69](image)
Also we found that the serum level of C3 was lower in group 1 and in group 2 than control group \((p=0.00009)\) \((p=0.0099)\) (Table 2), and was higher in group 2 than group 1 \((p=0.00005)\) (Table 3).

**Table (4):** Comparison between patients and control regard C3

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>SLE without CVD</th>
<th>SLE with CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>116.50</td>
<td>77.30</td>
<td>96.90</td>
</tr>
<tr>
<td>SD</td>
<td>18.95</td>
<td>5.44</td>
<td>6.19</td>
</tr>
<tr>
<td>Min</td>
<td>98</td>
<td>66</td>
<td>89</td>
</tr>
<tr>
<td>Max</td>
<td>153</td>
<td>84</td>
<td>109</td>
</tr>
<tr>
<td>T value</td>
<td>6.287242</td>
<td>3.1088057</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.000091</td>
<td>0.0099466</td>
<td></td>
</tr>
</tbody>
</table>

**Table (5):** Comparison between patients (group 1&2) regard C3

<table>
<thead>
<tr>
<th></th>
<th>SLE without CVD</th>
<th>SLE with CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>77.30</td>
<td>96.90</td>
</tr>
<tr>
<td>SD</td>
<td>5.44</td>
<td>6.19</td>
</tr>
<tr>
<td>Min</td>
<td>66</td>
<td>89</td>
</tr>
<tr>
<td>Max</td>
<td>84</td>
<td>109</td>
</tr>
<tr>
<td>T value</td>
<td>-7.52241</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.000005</td>
<td></td>
</tr>
</tbody>
</table>

**Fig (2):** Comparison between patients and control regard C3:

**Fig. (3):** CD expression on platelet from control one
DISCUSSION

SLE is a chronic multi system autoimmune disease with a broad range of clinical manifestation including photosensitive skin rashes, discoid lesions, arthritis, arthralgia, nephritis, cardiac and pulmonary disease and CNS disorder. The pathogenesis is attributed to circulating antinuclear antigens and dysfunction of T and B lymphocyte and dendrites cells\(^{(16)}\).

Patients with systemic erythematosis have a markedly increased risk to develop cardiovascular disease and traditional cardiovascular risk factor fail to account for this increased risk\(^{(17)}\).

Premature coronary heart disease (CHD) has emerged as a major cause of morbidity and mortality in patients with systemic lupus erythematosus (SLE). Overall SLE patients have a 5-6-fold increased risk of CHD and this excess risk is especially pronounced in younger women where the excess risk may be >50-fold. Risk factors alone do not completely explain the excess risk observed\(^{(18)}\).

The underlying mechanisms of increased risk of VD in SLE are unclear and the role of platelets not examined in this study we present strong association between CD69 in platelet and cardiovascular disease.

CD 69 is a marker of activation of inflammatory cells. It is suspected in pathology of vasculitis and possibly in platelet aggregation\(^{(11)}\).

In the present study we found that CD 69 expression on platelets were markedly higher in SLE patients with cardiovascular disease than
in healthy control and also we found that CD 69 expression are significantly higher in patients with SLE patients with cardiovascular disease than SLE patients without cardiovascular disease.

Consistent with that idea Christian et al.\(^\text{(16)}\) shows that CD 69 was found to be upregulated in platelets from SLE patients especially in patients with previous episodes of M I, and this also agree with Healy\(^\text{(18)}\) shows that CD 69 was the most significant probe set showing increased expression in myocardial infarction of allowed by MRP-14 protein, CD 69 is a constitutively expression protein on platelets and the platelets CD 69 m RNA level has been identified as a strong discriminator of acute st-segment elevation MI.

Although the role of C3 in atherogenesis remain unclear there is evidence that in the general population C3 levels correlate with waist circumference and postprandial lipemia suggesting a possible mechanism related to more traditional risk factors\(^\text{(19)}\).

Among our results C3 was significant decreased in SLE patients with and without myocardial infarction when compared to control group .in agreement with our finding Yang et al.\(^\text{(20)}\) reported lower C3 and C4 levels are traditionally associated with lupus pathogenesis and lupus activation .and also agree with Bao et al.\(^\text{(21)}\) suggested that low concentrations of complement components due to increased catabolism are found in majority of patients with active and severe SLE.

In this study also we found that C3 levels was increased in SLE patients with cardiovascular disease than SLE without cardiovascular disease and this agree with Manger et al.\(^\text{(22)}\) whom suggest that elevated C3 levels were predictive of coronary artery calcification and were more commonly found to be associated with symptomatic coronary heart disease in women with SLE and also agree with Trina et al.\(^\text{(23)}\) whom assume that higher serum C3 levels and immuno suppressant use at base line were related to progression of carotid intima media thickness and plaque in women with systemic lupus.

These finding suggest that Platelets with CD69 expression seem to more activated and may contribute to development of vascular complication in patients of SLE so CD69 expression on platelets may serve as predictive marker of CVD complication in patients with SLE.

**REFERENCES**

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تميل سي دي 19 في مرضى الذئبة الحمراء مع أمراض الأوعية الدموية والقلب

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أقسام التحاليل الطبية*الأمراض الباطنية**كلية طب البنات جامعة الأزهر.

أجري هذا البحث لقياس الكومبليمنت 3وسي دي 19 على الصفائح الدموية في المرضى الذين يعانون من مرض الذئبة الحمراء وتم تقسيم المرضى إلى مجموعتين:
1- مجموعة المرضى الذين يعانون من مرض الذئبة الحمراء (وكان عددهم عشرة مرضى)
2- مجموعة المرضى الذين يعانون من مرض الذئبة الحمراء مع أمراض الأوعية الدموية والقلب (وكان عددهم عشرة مرضى)

كما تم قص عشرة أشخاص أصحاء كمجموعة ضابطة، وقد تم فحصهم فحصاً أكلينيكيًا شاملًا وتم قياس مستوي الكومبليمنت 3وسي دي 19 على الصفائح الدموية.

وقد أوضحت النتائج أن سي دي 19 كان مرتفعًا في مرضى الذئبة الحمراء ويعانون من مضاعفات الأوعية الدموية والقلب بالمقارنة بالأصحاء وعند المرضى الذين يعانون من الذئبة الحمراء فقط كما وجده مستوي الكومبليمنت 3 كان منخفضًا في مرضى الذئبة الحمراء بالمقارنة بالأصحاء. ولكنها كانت مرتقعة في مرضى الذئبة الحمراء الذين يعانون من مضاعفات الأوعية الدموية والقلب.

ومن هذا البحث نستخلص أن سي دي 19 من الممكن أن يكون من دلائل من مضاعفات الأوعية الدموية والقلب في مرضى الذئبة الحمراء.