Study of LRRK2 and Beclin-1: Gene expression as autophagic markers in systemic lupus Erythromatosus in Egyptian populations

Eman Rizk EL Hassanein EL Fiky, Abeer Abdelmonaem Shahba, Hesham Ahmed El Serogy and Mohammad Abdelrahman Sweilam

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Abstract

Background: A severe form of heterogeneous autoimmune illness called systemic lupus erythematosus (SLE) is characterised by the generation of autoantibodies against specific self-antigens. The aim of the present study is to evaluate LRRK2 and beclin-1 as autophagic markers in patients of SLE and their role in the pathogenesis.

Methods: This case-control study was performed on 60 subjects, aged between 15-45 years old. Study participants were divided into two categories: Group I: comprised 30 patients with newly diagnosed SLE. Group II: included 30 apparently healthy age and sex matched subjects.

Results: SLEDIA was positively associated with beclin1, LRRK2, ANA, Anti ds DNA. Beclin1 was positively associated with LRRK2, ANA, Anti ds DNA. LRRK2 was positively associated with ANA, Anti ds DNA. Detection of beclin1 relative gene expression with 84% positive predictive value (PPV), 90% sensitivity, 83% specificity. Detection of LRRK2 its relative gene expression with 81% negative predictive value (NPV), 76% PPV, 73% sensitivity, 73% specificity.

Conclusions: These autophagic markers (LRRK2 and beclin1) correlate positively with the disease activity and severity and may have a role in disease pathogenesis and progression.

Keywords: LRRK2, Beclin-1, autophagic markers, systemic lupus erythromatosus

Introduction

Systemic lupus erythematosus (SLE) is a chronic, relapsing-remitting, multi-system autoimmune disease. The exact etiology is not known. The disease is presented with a wide variety of clinical manifestations due to autoantibody synthesis directed against nuclear antigens, serum proteins and cell surface proteins [1]. The disease is characterized by inflammatory changes, having a higher risk of morbidity in women between the ages of 15 and 45 than in men. The pathogenesis of SLE is complex involving genetic susceptibility, environmental factors, and hormonal factors [2].

Autophagy is a highly preserved process in which abnormal proteins, damaged organelles, foreign pathogens, and other cellular components are degraded via lysosome. Disruption of autophagy causes inhibition of ubiquitination, reduced mitochondrial function, accumulation of misfolded proteins, damaged organelles, and reactive oxygen species [3]. This causes defects in cellular homeostasis and development of many diseases like SLE [4].

Beclin-1 is a protein encoded by the BECN1 gene, which is located at a tumor susceptibility locus on chromosome (17q21.31). Beclin1 is the first identified protein in the mammalian autophagy pathway. It acts as an important regulator of autophagy through mediating the onset of autophagy and the maturation of the autophagosome through interactions with other proteins [5].

Leucine-rich repeat kinase 2 (LRRK2) is a large protein including multiple functional domains. LRRK2 is encoded by (LRRK2) gene which is located at chromosome 12q12 located in the Parkinson disease-8 (PARK8) loci. Missense mutations of this gene are the most common mutations present in familial autosomal dominant parkinson disease. LRRK2 can participate in different stages of autophagy such as autophagosome formation, autophagosome and lysosomal fusion and lysosomal functions through different mechanisms [6].
The current study's objective was to evaluate LRRK2 and Beclin-1 as autophagic markers in patients of SLE and their function in the pathogenesis of SLE and to study the correlation between these markers and the disorder severity and progression.

Patients and methods
The present case-control study was executed on 60 subjects selected from inpatient and outpatient clinic of Internal Medicine Department, Tanta University Hospitals and conducted in Clinical Pathology Department, Tanta University Hospitals from 2020 to 2022. Thirty newly diagnosed SLE patients were participated. All patients, who were between the ages of 15 and 45, met at least four of the 2015 American College of Rheumatology (ACR) SLE updated criteria.

After receiving approval from the Tanta University Hospitals’ Clinical Pathology Department’s replacement ethical committee, the study was carried out in Tanta, Egypt. Written informed consent was acquired from the patient. Exclusion criteria were the coexistence of other autoimmune conditions like rheumatoid arthritis, systemic sclerosis, pregnancy or lactation, the coexistence of chronic conditions that affect autophagy like systemic infections, cancers, diabetes mellitus, neurodegeneration, and heart conditions, etc., and the use of immunosuppressive medications within the previous two months.

Study participants were divided into two categories: Group I: comprised 30 patients of newly diagnosed SLE. The group includes 27 females and 3 males aged from 16 to 45 years old. Group II: included 30 apparently healthy subjects. The group includes 25 females and 5 males aged from 17 to 45 years old.

All patients and control groups were given a detailed history take and comprehensive clinical assessment including consensus guidelines provided by ACR, provide the basis for a diagnosis of SLE as per ACR criteria, either concurrently or consecutively, confirmed the diagnosis of SLE. Assessment of SLE activity was done. Routine laboratory investigations and antinuclear antibodies (ANA), anti-double strand DNA antibodies (anti-ds DNA), complement 3 (C3), complement 4 (C4), erythrocyte sedimentation rate (ESR), quantitative C-reactive protein (CRP) were measured measured. Specific investigations such as beclin1 (BECN1) and LRRK2 gene expression in peripheral blood mononuclear cell (PBMC) were evaluated utilizing quantitative-real time PCR.

PCR panels
Sample homogenization and lysis was the first step, then RNA binding, RNA wash, elution and synthesis of complementary DNA. The reaction mix (10 μl TOPreal™ 2X PreMIX (SYBR Green with low ROX)), 1 μl Template DNA or cDNA, 1 μl Primers 1 (5~10 pmol/μl), 1 μl Primers 2 (5~10 pmol/μl), 1 μl Primers 3 (5~10 pmol/μl), up to 20 μl Sterile water (RNase free) was prepared, the tubes were briefly centrifuged at room temperature. Qiang rotor gene 5 plex was programmed. The PCR tubes were placed in the real-time cycler and started the cycling program. The software included with Qiang Rotor Gene 5 plex was utilized for the initial data analysis in order to get raw CT results. The sequence of the primer pairs used were LRRK2 Forward: 5′-GAGCACGCTCCTCAAGTTTATTT-3′, Reverse: 5′-ACTGGCATTATGAACTGTTAGCA-3′. Beclin1 Forward: 5′-TGAGGGATGG AAGGGTCTAAG-3′, Reverse: 5′-GCCCTGGGCTGTGGTAAGTAATC-3′. house-keeping gene (GAPDH) Forward: 5′-GGAGCGAGATCCCTCCAAAAT-3′; Reverse: 5′-GGCTGGTGTGCATTCTCTATGG-3′

Statistical Analysis
The present study was statistically presented and analyzed using the following methods: Analysis of variance (ANOVA) tests (f), Mean value, Standard Deviation, and SPSS V.20 Fisher's precise test ROC curve, linear correlation coefficient, and chi-square.

Results
Regarding patients characteristics, age and sex were insignificantly different between groups. The systolic blood pressure (SBP) was significantly higher in SLE patients than in the control group. (P= 0.001*). Table (1)

<table>
<thead>
<tr>
<th>Table 1: Characteristics of the studied groups</th>
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<tbody>
<tr>
<td><strong>Patients group</strong></td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male (%)</td>
</tr>
<tr>
<td>Female (%)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
</tr>
</tbody>
</table>

Data are shown as Mean ±SD or frequency (%). SBP: systolic blood pressure. * p<0.05 is considered significant.

Haemoglobin, platelets count, C3 level and C4 level were significantly decreased in SLE patients contrasted to control group. The ESR level, CRP level, ANA level, Anti-ds DNA level, beclin1 level and LRRK2 level were significantly increased in SLE patients contrasted to control group. (Table :2).

<table>
<thead>
<tr>
<th>Table 2: Hematological and inflammatory markers, Renal assessment, LRRK2 of the studied groups</th>
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<tbody>
<tr>
<td><strong>Patients group</strong></td>
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<tr>
<td>Hb (g/dl)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Plt (10^9/ C.mm)</td>
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<tr>
<td></td>
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<tr>
<td>WBCs (10^9/ C.mm)</td>
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Patients were separated into 3 categories depending on the disease activity score. 23.3% have mild disease activity with SLE-DIA score (1-4), 53.3% have moderate disease activity with SLE-DIA score (5-10) and 23.3% have high disease activity with SLE-DIA score more than 10. Table (3)

Table 3: Systemic lupus erythematosus disease activity index (SLE-DIA) in patients group

<table>
<thead>
<tr>
<th>SLE-DIA grades</th>
<th>N (%)</th>
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<tr>
<td>Mild activity (1-4) score</td>
<td>7 (23.3%)</td>
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<tr>
<td>Moderate activity (5-10) score</td>
<td>16 (53.3%)</td>
</tr>
<tr>
<td>High activity (&gt; 10) score</td>
<td>7 (23.3%)</td>
</tr>
</tbody>
</table>

Data are presented as frequency (%). SLE-DIA: Systemic lupus erythematosus disease activity index

SLE-DIA was positively associated with beclin1, LRRK2, ANA, Anti ds DNA, and was negatively associated with C3 and C4 (P< 0.05). Beclin1 was positively associated with LRRK2, ANA, Anti ds DNA, and was negatively associated with C3, and C4 (P< 0.05). LRRK2 was positively associated with ANA, Anti ds DNA, and was negatively associated with C3, and C4 (P< 0.05). Table 4

Table 4: Correlation of SLE-DIA with other parameters in patients group

<table>
<thead>
<tr>
<th></th>
<th>SLEDA1</th>
<th>Beclin1</th>
<th>LRRK2</th>
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<tbody>
<tr>
<td></td>
<td>R</td>
<td>P value</td>
<td>r</td>
</tr>
<tr>
<td>Beclin1</td>
<td>0.710</td>
<td>0.001*</td>
<td>-</td>
</tr>
<tr>
<td>LRRK2</td>
<td>0.637</td>
<td>0.001*</td>
<td>0.600</td>
</tr>
<tr>
<td>ANA</td>
<td>0.638</td>
<td>0.001*</td>
<td>0.469</td>
</tr>
<tr>
<td>Anti ds DNA</td>
<td>0.780</td>
<td>0.001*</td>
<td>0.579</td>
</tr>
<tr>
<td>C3</td>
<td>-0.525</td>
<td>0.003*</td>
<td>-0.637</td>
</tr>
<tr>
<td>C4</td>
<td>-0.390</td>
<td>0.033*</td>
<td>-0.518</td>
</tr>
</tbody>
</table>

Table 4: Correlation of SLEDA1 with other parameters in patients group

Discussion

Systolic blood pressure level was significantly elevated in SLE group contrasted to the level of the control group. This agreed with several studies which reported increased level of SBP in SLE than normal persons suggesting association between inflammatory markers and elevated SBP in SLE group [10-12].

Also, the hemoglobin (Hb) concentration was significantly reduced in SLE group contrasted to the level of the control group. This was in consistent with many studies which found lower levels of Hb concentration in SLE in comparison to normal persons. They explained that by the effect of cytokines on iron hemostasis and erythropoietin production [10, 11, 12]. On the other hand, Uzkeser et al. [13] said that Hb levels between the SLE group and control group did not differ significantly.

Using the ROC curve, 1.15 was set as cutoff value for detection of beclin1 relative gene expression with 89% NPV, 84% PPV, 90% sensitivity, 83% specificity and 0.904 area under curve (AUC) as given in (Figure 1).

Fig 1: ROC curve analysis of beclin1 and LRRK2 relative expression in studied groups

Using the ROC curve, 1.15 was set as cutoff value for detection of beclin1 relative gene expression with 89% NPV, 84% PPV, 90% sensitivity, 83% specificity and 0.904 area under curve (AUC).

As regard LRRK2, 1.1 was set as cutoff value for detection of its relative gene expression with 81% NPV, 76% PPV,
group contrasted to the levels of the control group, while Cylwik et al. [10] and Uzkeser et al. [13] observed no difference between the control group and patient group that was significant.

This study showed that ESR was significantly increased in SLE group compared to control group. This agreed with several studies which reported higher ESR in SLE group than normal persons, which might be due to increased fibrinogen in the acute phase response of the disease activity [10, 11, 13].

This present study showed a significant increase in CRP level in SLE group than control group. This agreed with many studies which reported higher CRP in SLE group than normal persons [10, 11].

Regarding Immunological markers, ANA level was significantly increased in SLE group contrasted to control group. ANA level was positively associated with SLE disease activity index ($r=0.638; P= 0.001$). This was in accordance with Liu et al., [16] and Qu et al. [17] who reported elevated serum ANA level in SLE group than normal persons and it was considered as an entry criterion in order to categorise SLE. This increased production of ANA was suggested to be due to loss of B cell tolerance, followed by the identification of self-antigens and development of autoreactivity.

The anti double stranded DNA antibody (Anti ds-DNA) level showed a significant rise in SLE group in comparison to control group. The SLE disease activity index and anti-ds-DNA level showed a positive correlation. This was in accordance with Qu et al. [17] who reported elevated serum Anti ds-DNA level in SLE group than normal.

As regards the complement 3 (C3) and complement 4 (C4) levels, the present study showed a significant decrease in SLE group in comparison to control group. C3 and C4 levels were negatively associated with SLE disease activity index. This was in accordance with Rana et al. [18] Weinstei et al. [19]

The BECN1 expression was substantially increased in SLE group contrasted to control group. Using the ROC curve, 1.15 was set as cutoff value for detection of beclin1 relative gene expression with 90% sensitivity and 83% specificity. Beclin1 expression was positively associated with SLE disease activity index. This agreed with Khalil et al. [20], Tanaka et al. [21], Raj et al. [22]

Skibo et al. [23] has demonstrated that beclin1 and Vps34 revealed that SLE patients’ T cells have more protein than normal. In mammals, the beclin1 (Atg6) gene is essential for autophagy. By joining the Vps34 -PI3K-CIII complex, the protein that is produced by this gene promotes the development of autophagosomes and is actively implicated in the onset of autophagy. We conclude that autophagy is triggered in SLE patient T cells because both proteins are components of the beclin 1-Vps34-Atg14-P13P complex, which is necessary for the elongation of pre-autophagosomes.

This study revealed that beclin1 was positively correlated with SLEDIA, LRRK2, ANA and Antids DNA. This study showed a negative correlation of beclin1 with C3 and C4. This agreed with [6] who reported negative correlation of beclin1 with C3, but they reported no significant correlation of beclin1 with C4.

Kaur and Changotra [24] have demonstrated the existence of several factors and alterations that control how beclin 1 interacts with other proteins to fulfil various functions in cells. These beclin 1 changes at the gene and protein levels are very significant because they have the ability to directly influence beclin 1 and the function it performs. Therefore, beclin 1 may be a viable pharmacological target that might be utilised alone or in combination with other medications to treat a variety of disorders, including SLE.

This study showed that the LRRK2 expression was significantly increased in SLE group contrasted to control group. Using the ROC curve, 1.1 was set as cutoff value for detection of LRRK2 relative gene expression with 83% sensitivity, 73% specificity. LRRK2 expression was positively correlated with SLE disease activity index. This agreed with Gan and Zhou [25]

This study revealed positive correlation between LRRK2 and SLEDIA, beclin1, ANA and Antids DNA. This agreed with Zhang et al. [26] who reported positive correlation of LRRK2 with SLEDIA and IgG level.

LRRK2 was negatively correlated with C3 and C4. This agreed with Zhang et al. [26] who reported negative correlation of LRRK2 with C3 and C4 as their decrease correlates with increased disease severity. Zhang et al. [26] have shown that the SLE group's B cells have abnormally high levels of LRRK2 expression. SLEDAI, C3 and C4 levels, which were important markers for the diagnosis of SLE, were linked with this aberrant expression of LRRK2 in B cells. Additionally, LRRK2 inhibitor therapy seems to encourage LRRK2's ubiquitination, which in turn speeds up its proteasomal destruction. Further research is necessary to determine whether the LRRK2 inhibitor now available can specifically delay B cell activation and differentiation for disease remission in light of the rise in LRRK2 expression in B cells and its role in the pathogenesis of SLE. [26], ROC curve analysis between studied groups showed larger total area under the curve (AUC) value, which represents the overall ability of the medical exam to appropriately differentiate between the two scenarios. The levels of beclin1 and LRRK2 may therefore be useful biomarkers for differentiating between SLE patient and control groups, as indicated by the total area under the ROC curves.

Conclusions

The current study showed increased expression of autophagic markers (beclin1 and LRRK2) in SLE patients contrasted to apparently healthy age and sex matched controls. This suggested that these autophagic markers could contribute to the pathophysiology of SLE. These autophagic markers correlate positively with the disease activity and severity and may have a role in disease progression.

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Conflict of Interest: Nil

References


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