ASSESSMENT IMMUNOLOGICAL TEST WITH C- REACTIVE PROTEIN IN A NEWLY DIAGNOSED SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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ABSTRACT

Background: Due to disease heterogeneity, low sensitivity, specificity of immunological tests, and other factors that make diagnosing people with Systemic lupus erythematosus (SLE) difficult and then monitoring their disease activity. The C-reactive protein (CRP) is a prototypical acute phase protein in humans, which rises rapidly in response to inflammation and some autoimmune diseases, but not in SLE flares. The role of CRP in SLE remains controversial. However, CRP enhances the clearance of apoptotic cells, an effect that likely contributes to homeostasis in systemic autoimmune diseases.

Aim of the study: This study aimed to examine the relationship between CRP and newly diagnosed SLE patients through immunological tests such as Antinuclear Antibodies (ANA), double-stranded DNA (dsDNA), complements C3, C4. And CRP levels were determined to distinguish between disease exacerbations and inflammation in SLE patients when compared with the Erythro Sedimentation Rate (ESR).

Patients and Methods: Eighty-eight patients with SLE according to the classification criteria of the European League Against Rheumatism / American College of Rheumatology (EULAR / ACR) and 70 healthy controls were collected in this study. To carry out the blood analysis, a five-millimeter sample was taken from all subjects.

Results: The results show that CRP, ESR, ANA, dsDNA, C3 and C4 showed a significant difference in SLE patients than in healthy controls. We investigated a significant correlation between CRP only with ESR, ANA, and C3.

Conclusion: In summary, the C-reactive protein blood test is a non-specific-marker for lupus disorders and is associated with the ANA, ESR and C3 variants.

Keywords: systemic lupus erythematosus, erthro sedimentation rate, antinuclear antibodies, C-reactive protein, C3, C4

I. INTRODUCTION

The systemic lupus erythematosus (SLE), is an autoimmune disease characterized by abnormal production of a wide and heterogeneous range of autoantibodies. It will mainly affect females in puberty and menopause. The incidence ratio of female/male is 9/1. If the diagnosis of SLE is delayed, serious damage occurs in vital organs of the body [1]. The complicity of this disease appeared in its effect on clinical features such as renal, neurological disorders or inducing arthritis [2]. It is also associated with an increased risk of premature death [3]. In patients with SLE, distinguishing between infection and disease activity might be difficult, doing so will benefit in medication decisions. With increasingly sensitive means of testing for CRP, it is worth knowing whether CRP may help distinguish infection from active disease in patients with SLE.
C-reactive protein serum level is frequently related with the level of IL6 and is routinely assessed as a marker of inflammation in a range of disorders. However, despite elevated IL6, CRP often decreases in autoimmune illnesses (SLE).4

It is one of the proteins at the acute phase.[5]–[7]. Authors have been reported that the CRP levels affected by many parameters such as age, body mass index (BMI), infection, medications, cardiovascular accidents and malignancy [8]–[10]. The CRP role in the diagnosis of SLE remains unclear [11], [12].

The ability of CRP to accelerate the clearance of apoptotic cells as well as its tendency to bind to nuclear antigens led to the idea that CRP could prevent autoimmunity by masking or promoting self-antigens from the immune system.2

Generally, In SLE especially in its systemic form, autoantibodies directed to nuclear (ANAs), cytoplasmic, and cellular membrane antigens are considered the serological hallmark. [13]. These nuclear antigens include single strand (ss) and double strand (dsDNA) (deoxyribonucleic acid), histone proteins, nucleosomes (histone-DNA complex), (Smith antigen (Sm), etc.) [14].

Rapid and accurate flare-up assessment of SLE is essential in routine patient care and in lupus clinical trials, where time to first flare-ups and frequency and severity of episodes are common major secondary end points. Clinical acumen, detailed history, along with targeted laboratory testing, are the cornerstones of the evaluation of a lupus flare, with a secondary role for serological markers 240

Among other autoantibodies, complement activation has demonstrated pivotal in the pathogenesis of SLE-associated immune complex damage. The complement system is a complex pathway in the immune system consisting of proteins and receptors that improve the ability of antibodies and phagocytes to remove microbes and damaged cells from an organism. In addition to its function in fighting infectious diseases, it also plays a key role in the inflammatory response induced by immune complex deposition especially in SLE.8

Complement is activation through three pathways: classical, alternative and lectin pathways. While complement deficits may play a role in the onset of SLE, complement dysregulation may also be present during the course of the disease. As a result, the supplement is now used as a biomarker for SLE.7

The presence of foreign invaders triggers the C3, C4 protein to be cut (cleaved) into two smaller pieces. One of these pieces, called C3b, C4b interacts with several other proteins on the surface of cells to trigger the complement system response. This process must be carefully regulated so the complement system targets only unwanted materials and does not damage the body's healthy cells. SLE is a disease that often causes abnormal C3 and C4 [15].

Involvement of the complement system in SLE has been described by several researchers over the past 70 years with low levels of complement proteins (C3, C4) and hemolytic activity that can be used as diagnostic markers for SLE and for monitoring disease activity.9

However, inherited deficiencies in the complement system show an independent predisposition of affected individuals to bacterial infection and SLE.8

The limitations of C3 and C4 as biomarkers to diagnose and monitor SLE activity prompted the development of assays to measure the proteolytic fractions of complement proteins. Since these fragments are formed upon complement cascade activation, the cleaved complement products reflect complement activation more accurately than levels of intact individual proteins.9

A very important property of CRP is the ability to bind C1q to activate the classical complement cascade. Complement activation by CRP differs from activation by antibody in the presence of selective activation of early components without formation of the membrane attack complex (MAC).2

Erythrocyte sedimentation rate (ESR) is an inflammatory marker of SLE activity. 34m Inflammation can cause an increase in CRP and ESR, which can be caused by autoimmune diseases, infection, or cancer. SLE patients had a higher incidence of elevated ESR from CRP patients than from rheumatoid arthritis patients, although elevated
ESR was associated with both lupus activity and infection, making the distinction between lupus and infection unclear. On the other hand, ESR elevations are closely associated with SLE exacerbations.

This study aimed to evaluate the C-reactive protein serum level in newly diagnosed SLE patients to distinguish between flare-ups and infections and to determine the abnormal CRP levels during active immunological processes and their impact on SLE compared with ESR makes it a valuable tool for both initial treatment selection and long-term disease management. Also, to investigate the association between polymorphisms CRP receptors and cytokines that promote CRP synthesis that regulate CRP production to influence disease risks.

Patients and Methods

This is a case-control study conducted in Rheumatology Unit /Baghdad Teaching Hospital, Baghdad medical city, Baghdad, Iraq. Eighty-eight patients were included in this study with new diagnostic of SLE according to the European League Against Rheumatism/ American College of Rheumatology (EULAR /ACR) classification criteria of SLE. Seventy healthy volunteers were included as a control sample. All subjects underwent a questionnaire and ethical informed consent. This study was approved by the ethical committee of the College of Medicine/Al-Nahrain University International Review Board (I.R.B).

A 5 ml of venous blood were drawn from all subjects using a single-use syringe, 2ml out of them was transferred to the ethylene diamine tetraacetic acid (EDTA) tube for ESR test (AFCOVAC, Spain). A 3 ml out of 5 ml of drawn blood was dispensed into a vacuum tube (gel tube manufactured by DhawAl-Qammar, Sharjah/ UAE), then it stays at room temperature for 15 minutes to clot. Finally, it's centrifuged (using Rotofix 32A centrifuge, German) at (13,000 rpm) over 20 minutes. These prepared samples were used to measure the laboratory tests: ANA, and dsDNA kit manufactured by Aesku, Germany, C3 and C4 kit manufactured by LTA, Italy, while CRP kit manufactured by Roche, USA).

Statistical analysis was performed using SPSS version 24. An unpaired t-test was used to find the significant difference between the two groups. A person correlation test is used to test the correlation between the CRP and other parameters. The significant p-values were 0.05.

I. RESULTS

The characteristics of patients and healthy subjects are shown in table (1). The mean age of SLE patients was 30.95 ± 8.25 years and controls were 29.97 ± 8.06. There is no significant difference in age between SLE and control. We found a significant difference in the gender of subjects in this study. Figure (1) shows the gender distribution for all subjects where 74 (92.5%) of SLE patients were female and 6 (7.5%) of them were male. The female in the control group was 64 (91.5%) and the male was 6 (8.5%).

Table (1): the characteristics of systemic lupus patients' erythematous and control subjects.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Systemic lupus erythematous</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.95 ± 8.25</td>
<td>29.97 ± 8.06</td>
<td>0.4522</td>
</tr>
<tr>
<td>Gander</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>92.5%</td>
<td>91.5%</td>
<td>&lt;0.00001*</td>
</tr>
<tr>
<td>Male</td>
<td>7.5%</td>
<td>8.5%</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>26.80 ± 4.38</td>
<td>26.04 ± 4.01</td>
<td>0.2558</td>
</tr>
</tbody>
</table>

* Significant difference at the 0.05 level.
Table (2) and figure (2) summarize the analysis of parameters used in this research for testing the significant difference between the SLE and healthy people. The difference in all parameters (ESR, CRP, ANA, dsDNA, C3 and C4) between the two groups was statistically significant.

The serum levels of ESR, CRP, ANA, and dsDNA for SLE patients were higher than control. While the C3 and C4 levels were lower than normal.

Table (2): the analysis of systemic lupus patients’ erythematosus and control subjects' parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Systemic Lupus Erythematosus</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>54.32 ± 3.10</td>
<td>13.42 ±5.34</td>
<td>0.00001*</td>
</tr>
<tr>
<td>CRP</td>
<td>2.92 ± 0.47</td>
<td>1.45 ±1.02</td>
<td>0.0006*</td>
</tr>
<tr>
<td>ANA</td>
<td>80.36 ± 25.48</td>
<td>2.33±2.29</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>dsDNA</td>
<td>29.93 ± 11.62</td>
<td>0.35 ±0.02</td>
<td>0.000023*</td>
</tr>
<tr>
<td>C3</td>
<td>45.01 ±28.15</td>
<td>125.92 ±19.17</td>
<td>0.0001*</td>
</tr>
<tr>
<td>C4</td>
<td>11.52 ±2.58</td>
<td>35.57 ±8.05</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

* Significant difference at the 0.05 level.

Figure (1): The Gender Distribution for All Subjects.

Figure (2): The Parameters Comparison Between the SLE patients and Healthy Control Subjects.
The correlation results between the CRP and parameters included in this study for SLE patients are given in Table 2. There was a correlation between the CRP and ESR, ANA, and C3. There is a correlation relationship between the ERS and CRP and is given by \( r_s = 0.22389 \), \( p\)-value = 0.036. Furthermore, the ANA correlation value is given by \( r_s = -0.27872 \), \( p\)-value = 0.00855. The dsDNA shows no correlation at \( r_s = 0.13344 \), \( p\)-value = 0.21519. The C3 correlation value is \( r_s = -0.26058 \), \( p\)-value = 0.0142. Other variable such as C4, did not show any significant correlation with \( r_s = -0.12966 \), \( p\)-value = 0.22858.

Table (3): the correlation of CRP and systemic lupus patients’ erythematosus parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( r_s )</th>
<th>( p)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>0.22389</td>
<td>0.036*</td>
</tr>
<tr>
<td>ANA</td>
<td>-0.27872</td>
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</tr>
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<td>dsDNA</td>
<td>0.13344</td>
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<tr>
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<td>-0.26058</td>
<td>0.0142*</td>
</tr>
<tr>
<td>C4</td>
<td>-0.12966</td>
<td>0.22858</td>
</tr>
</tbody>
</table>

* Significant difference at the 0.05 level.

III. DISCUSSION

Lupus is an autoimmune disease that affects the kidneys, joints, blood vessels, skin, heart, lungs, and brain, among other tissues and organs [17]. This research attempt to find the linkage between the C-reactive protein and the SLE disorder. The ability to precipitate the Streptococcus pneumonia cell wall called C-polysaccharide has been attributed to CRP activity. This activity, known as CRP while other activities such as apoptotic cell clearance, activation of complement, and binding to various autoantigen, including chromatin, histones, and another antigen, are controversial. Szalai AJ pointed it out in 2004. That the CRP could mediate protection against autoimmunity [5]. Several studies have explored that the serum levels of CRP increased as the time of SLE disease increase [18]. This is a matter of opinion with which the author of previous papers agrees with the finding of this study.

S. Kakati et al. almost agreed so in this study, they found the CRP levels in SLE patients are higher than in the control group, but the results are not significant [19].

Barnes et al. [20], in agreement with this study, found a positive correlation between highly sensitive CRP and C3 values. They proposed that C3 is an acute phase reactant and increases as CRP. Also, E Littlejohn. et al. [21] found that higher CRP levels were found in SLE patients, claiming that CRP levels were twice as high in patients with pneumonia compared to those with any other type of SLE. CRP is a plasma serum protein that is secreted by human hepatocytes in response to the sixth interleukin during inflammation of the body.

The ESR shows to correlated with the CRP in our study. The ESR may provide primary guidance to inflammation [22].

They directly correlated the sedimentation rate with the concentration of acute-phase proteins, particularly fibrinogen, in the plasma that causes aggregation of erythrocytes [22]. The rate of rising fibrinogen in the acute-phase response may be slower than that of CRP. Therefore, because of this indirect method of measurement, the ESR is influenced by multiple factors, including the concentration of plasma proteins and the concentration, size, and shape of erythrocytes. Both measures also vary in the population: ESR rises with age and is higher in women compared to age-matched men and CRP can vary with sex and race [23].

The large response of inflammation and involvement of the serosal membrane component may cause an elevation of the CRP level. For that reason, the CRP levels vary.

In this work, we observed a significant negative correlation between C3 with the CRP. While we found no significant correlation with C4. The SLE patients show to have lower C3 and C4 values than healthy subjects. When the immune system becomes activated (with autoimmune diseases), the complement system may become involved in the attack. This can reduce the levels of complement as it is consumed in autoimmune attacks. The C3 and C4 behavior are paradoxical, especially in the acute phase of lupus [15], [21], [24].
In our result, the ANA levels in SLE patients show a higher significant value than the normal subjects. The ANA serum is one of the SLE markers for diagnosis of the diseases and monitoring its progression [25], [26]. It plays an important role in promoting immunity dysregulation and tissue injury through ingestion with the dead lupus cell results as an immune response. These complexes activate the immune system and cause the production of antibodies and inflammation [24]. There is a strong negative correlation between the CRP values in this study. So, the ANA levels varying as a response to CERP variation and vice versa.

The dsDNA is significantly higher in lupus patients. In addition, it shows no correlation with the CRP. But the dsDNA does not always produce the SLE….. These can arise from changes in the environment or inheritance. Hypo methylation, especially in T cells, can cause SLE [27].

The ANA and dsDNA are antibodies released in a higher-levels when an inflammation attacks the body as a response to increasing the immunity of the body. While dsDNA and ANA may be present at a low level with several disorders, it is high primarily associated with lupus. Lupus is strongly associated with a high level of dsDNA in the blood and is often significantly increased during or just before a flare-up. When the dsDNA is positive and the person tested has other clinical signs and symptoms associated with lupus, it means that the person tested likely has lupus. Normally, antibodies protect against infection and are produced when a person’s immune system fails to adequately distinguish between “self” and “non-self.” They mistakenly attack the body’s healthy cells, causing tissue and organ damage. The dsDNA specifically targets the genetic material (DNA) found in the nucleus of a cell, hence the name "dsDNA." The dsDNA test identifies these autoantibodies in the blood [17].

Kumar Y. and Bhatia A. [28] investigated the role of ANA and led to dsDNA changes in SLE disease. They found that SLE excreted higher levels of ANA than normal people. They destroy the molecular structure and are sometimes categorized as strands of DNA.

IV. CONCLUSION

In conclusion, The C-reactive protein is a valuable and sensitive marker to predict lupus disorders and is associated with their sedimentation rate, antinuclear antibodies and complement C3 proteins.

REFERENCES


