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The Assessment of Complement Proteins in Patients With Severe COVID-19

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Abstract

Introduction: The complement system, consisting of more than 20 soluble proteins, has a crucial role in innate immunity and inflammation and eliminates pathogens and viral infections. Therefore, we investigated the titers of C3, C4, and total IgG in the sera of non-severe and severe coronavirus disease 2019 (COVID-19) patients.

Methods: For this purpose, peripheral blood samples were collected from 30 non-severe and 30 severe COVID-19 patients and 30 healthy individuals with similar age and sex as the control group. The serum levels of total IgG, C3, and C4 were analyzed. Also, white blood cells, platelets (PLTs), and lymphocytes were counted by an auto-analyzer.

Results: White blood cell count showed no difference between COVID-19 patients and the control group. The results showed a significant decrease in lymphocyte and PLT counts in COVID-19 patients compared to the control. Complement proteins, including C3 and C4, were increased in non-severe COVID-19 patients (C3; $P=0.017$ and C4; $P=0.012$) compared to other groups. Total IgG showed a notable decrease in severe COVID-19 patients.

Conclusion: The decrease in C3 and C4 complement factors in severe COVID-19 patients may be due to further consumption secondary to the formation of immune complexes. By clarifying the role of complement proteins of C3 and C4 in different stages of the disease, our results can be helpful in designing therapeutic and diagnostic measures for the disease.

Keywords: COVID-19, Complement, C3, C4, IgG, Inflammation

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Introduction

The novel coronavirus 2019 (COVID-19) belongs to the family of Coronaviruses.[1] The global outbreak of COVID-19 started in Wuhan city, China, and now is known as a significant threat to the public health systems.[2] According to the pandemic of coronavirus that rises daily worldwide, any researcher's effort is dedicated to overcoming the virus. Therefore, there is an urgent need to understand the immune response to COVID-19 better until we can find the best treatment and management of the disease.[3]

The most important reaction to control and resolve the viral infection is the effectual host immune response, including innate and adaptive immunity; however, the severity and outcome of the COVID-19 might be associated with the excessive immune response.[4-6] In this respect, the complement system has a vital role in the innate immune system and contributes to the systemic inflammation against different pathogens. The complement system uses various mechanisms such as enhancing humoral immunity, regulating antibody effector mechanisms, and modulating T-cell function by linking the innate and adaptive immune responses. The complement system is activated in three general pathways: classical, alternative, and lectin, with C3 and C4 molecules.[7]

The C3 protein has a central role in the complement cascade, and its investigation could be an appropriate criterion, indicating the activation of three pathways of complement.[8] The C4 protein has a

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crucial role in the immune response via the classic and lectin complement pathways. Complement neutralization exerted with C4 by enveloped viruses had been shown in past studies. [9] However, a recent study suggested that the presence of an unknown C4-dependent mechanism blocks the infection of non-enveloped viruses. [10] Furthermore, previous studies showed that gene polymorphisms of mannose-binding lectin, which plays an essential role in the lectin pathway, were significantly associated with evere acute respiratory syndrome coronavirus (SARS-CoV) infection susceptibility.[11,12] Here, we measured the complement proteins (C3 and C4) and total IgG antibody concentration in severe and non-severe COVID-19 patients.

Materials and Methods

Patients

Ninety unrelated subjects participated in this case-control study. COVID-19 patients were categorized into 30 severe patients (14 females, 16 males) and 30 non-severe patients (13 females, 17 males, and 30 healthy individuals (15 females, 15 males) with similar age and sex were included as the control group.

Inclusion and Exclusion Criteria

All patients were initially recognized based on the clinical manifestation and ultimately by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of throat swab samples. The COVID-19 mild group included the patients who had oxygen saturations higher than 95% during the disease follow-up and did not require Intensive care unit (ICU) admission. Blood samples were gathered on the day of admission. COVID-19 severe group considered those patients with oxygen saturations lower than 93%, and arterial blood oxygen partial pressure (PaO2)/oxygen concentration (FiO2) ≤ 300 mm Hg and had intubation and admission to the ICU needed, and blood samples were gathered within 48 hours after admission to the ICU. In the control group, healthy individuals without a history of severe illness or infection during the past month were used. 5 ml of blood was taken from each participant and serum separated by centrifuge and immediately stored at −80°C until analysis. We excluded the patients who received immunosuppressive therapies in the past and those who died of the disease. The samples were collected according to the laboratory testing of humans suspected of novel coronavirus infection guidelines.

Blood Cells' Measurements

Whole blood samples were collected from patients and healthy individuals. Blood specimens were analyzed for counting white blood cells (WBCs), platelets (PLTs), and lymphocytes by a hematology auto-analyzer (Sysmex, KX-21N).

Nephelometry

Measuring C3, C4, and total IgG was performed by the Nephelometry assay according to the manufacture's instruction (The Binding Site Group Ltd., UK). The evaluation of soluble antigen concentration by the nephelometric method involves a reaction with specific antiserum to make insoluble complexes. Concentrations are automatically calculated by reference to a calibration curve that existed within the instrument. Briefly, serum samples were diluted with buffer, and antiserum was added to the samples and mixed well. The absorption was immediately recorded by a MININEPH PLUS device, and C3, C4, and IgG concentrations were measured using a standard curve.

Statistical Analysis

Statistical analyses were performed in SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Data presented as mean ± standard deviation. One-way ANOVA with Tukey post hoc test was used for comparison of means between the groups. A P value of < 0.05 was considered statistically significant.

Results

WBCs and PLTs Evaluation

WBCs count showed no changes between the studied groups ($P < 0.05$) (Figure 1A); however, PLTs were decreased significantly in severe COVID-19 patients compared with healthy individuals ($P = 002$) and non-severe COVID-19 patients ($P = 0.031$) (Figure 1B). Also, the results showed a significant ($P = 0.024$) increase of polymorph nuclear leukocytes in severe COVID-19 patients than the other groups (Figure 1C). Furthermore, lymphocytes were decreased significantly in severe and non-severe COVID-19 patients (Figure 1D).

Complement C3 and C4 Proteins and Total IgG

Examination of total IgG in the serum samples by nephelometry was showed a significant decrease in severe COVID-19 patients ($P = 0.037$; Figure 2A). Complement proteins, including C3 and C4 detection in the serum samples, confirmed increased concentration levels of C3 ($P = 0.017$) and C4 ($P = 0.012$) proteins in non-severe COVID-19 patients than the healthy individuals. Also, the results showed a significant decrease in C3 and C4 proteins in severe COVID-19 patients than non-severe COVID-19 patients (P = 0.014; Figure 2B-C).

Discussion

The most critical responses to viral infections are the innate and acquired immune systems. Innate immune responses include the use of macrophages and neutrophils, activation of the complement system, and production of antimicrobial peptides. The complement system uses various mechanisms to remove pathogens.[4] In this study,

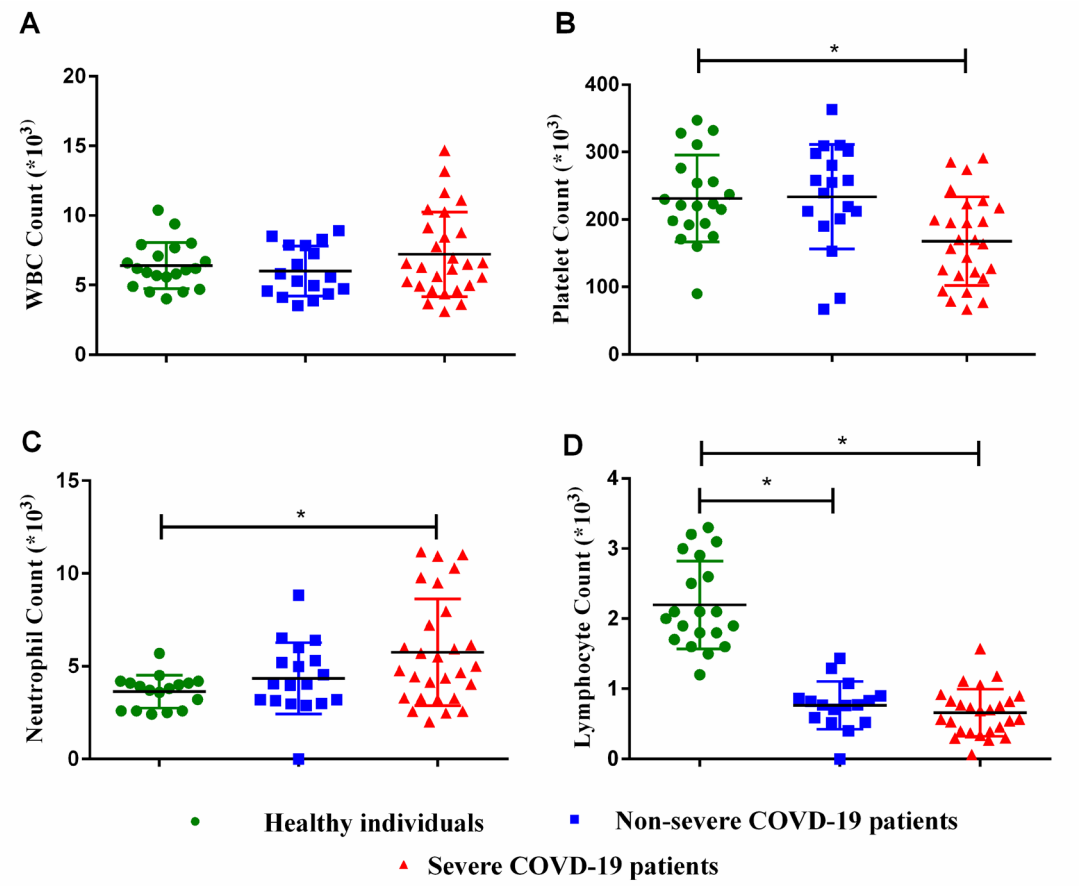

Figure 1. Blood Cells Analyzing in Severe and Non-severe COVID-19 Patients. (A) WBC count, (B) Platelet count, (C) Neutrophil count, (D) Lymphocyte count. *$P<0.05$.

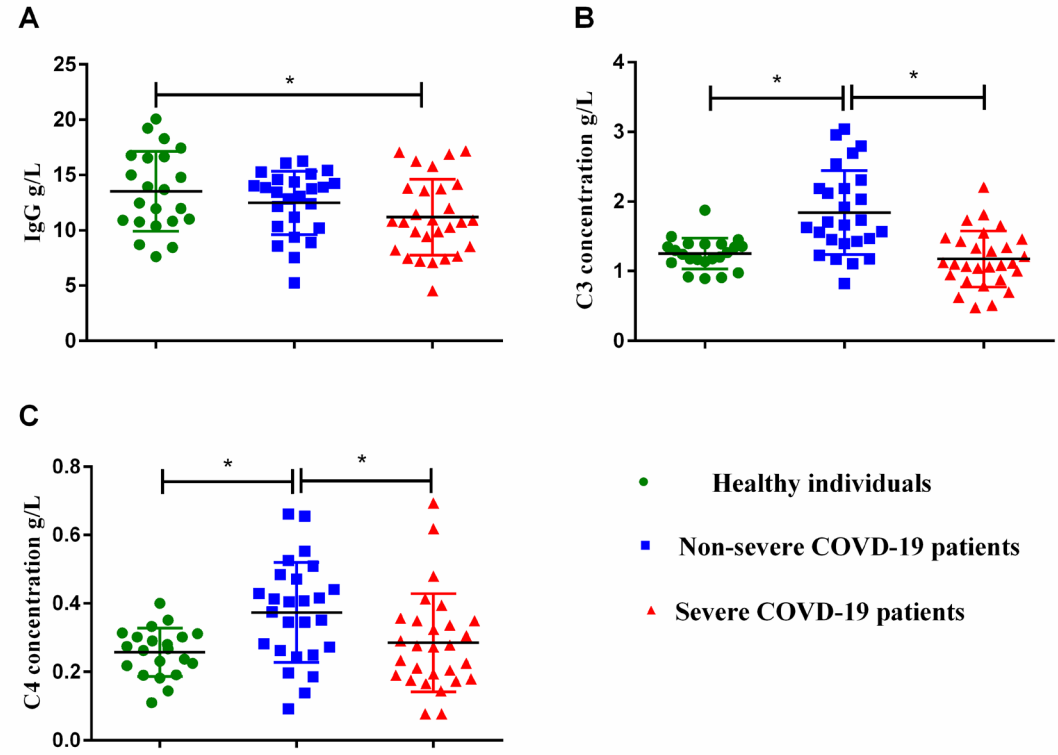

Figure 2. Nephelometry Analysis Results of C3, C4, and Total IgG Antibodies in Three Investigated Groups. (A) Total IgG, (B) C3 level, (C) C4 level. *$P<0.05$.

we evaluated blood cell count, C3 and C4 complement factors level, and total IgG in patients with severe and non-severe COVID-19 patients.

Examination of white blood cell counts showed no difference in WBC levels among the studied groups, whereas patients with severe and non-severe COVID-19 had lymphopenia. In parallel, a study of 108 patients with COVID-19 reported that the WBC count was not different in 97% of patients compared to healthy individuals. In contrast, 67% of patients had lymphopenia, which was confirmed in COVID-19 infection in the past studies.[5,13]

In this study, the number of platelets counted in patients with severe COVID-19 was reduced compared to the other two groups, and the patients had thrombocytopenia. Previous studies have shown thrombocytopenia in HIV-infected patients.[14] A study of 1476 patients with COVID-19 in China found that 238 patients (16.1%) had died and 306 (20.7%) had thrombocytopenia. From these studies, it can be concluded that thrombocytopenia is common in patients with COVID-19 and is associated with increased mortality risk.[5,15]

Also, our results showed neutrophil count in patients with severe COVID-19 increased compared to the other groups. Neutrophils can be increased due to the inflammatory cytokines and chemokines, including interleukin (IL)-8 and IL-6 produced from the innate immune system.[6,16]

IgG total titers decreased in patients with severe disease compared to non-severe patients and healthy individuals. A study by the Zhe Du group of 60 patients recovering from COVID-19 showed that antibody detection could indicate COVID-19 progression. Also, past studies showed antibodies could be reduced in COVID-19 patients. Patients' antibody levels and clinical manifestations indicate that antibody titer detection could predict the severity of the disease.[17] Also, in another study on 38 patients in the acute phase of SARS- CoV- 2 infection, IgM and IgG titer have been reported to be negative in 31 of them,[18] which could be the reason for the decrease in total antibody in people with severe COVID-19.

In this study, the C3 and C4 factors in the group with moderate disease form increased significantly compared to the control group and patients with severe disease form. There was also a significant reduction in complex C3 and C4 proteins in the group of patients with severe COVID-19 disease compared with non-severe patients. Li and colleagues conducted a study on patients with SLE and RA and examined serum levels of C3 and C4 factors. The results showed that C3 and C4 complexes in patients with SLE gradually decreased with increased disease activity.[19]

Measurement of serum C3 and C4 factors help diagnose and monitor immune complex diseases such as SLE and some blood-related infectious diseases. Complement is the same as acute-phase proteins, and it may be normal to complement some inflammatory and infectious disorders. C3 and C4 are measured simultaneously, indicating the activation of both the classical and alternative complement pathways. Therefore, due to the activation of C3 alone, the concentration of this component in some infectious diseases (septicemia, endocarditis) is reduced. The C3 and C4 titers are often reduced in immune complex disorders. For example, C4 alone is diminished as a diagnostic marker in angioedema, immune complex diseases, especially vasculitis and cryoglobulinemia, and cold agglutinin. [20]

In addition, in another study which was done on the C3 level in Wuhan, levels of serum amyloid A, C-reactive protein, and lactate dehydrogenase were measured. They also concluded that the level of complement C3 was increased, which is associated with the risk of developing severe COVID-19, specifically in young patients. Their result showed that the amount of C3 in patients could be influenced by age, gender, and comorbidities. The increased level of C3 is considered a unique risk factor for adverse outcomes, especially to young people.[21]

Overall, all the observations are indicated that, like the other pathogenic viral diseases, COVID-19 is a complement-mediated disease, and complement cascade maybe act as an effective candidate for medical intervention to reduce the severity.[22] The other researches have shown that the level of complement C3 is often fallen during infections due to consumption, to the reduction of C3 and C4 is shown in immune complex disease.[23,24]

In conclusion, C3 and C4 complement factors increase in non-severe COVID-19; however, in the severe COVID-19 patients, the complement proteins will be consumed by forming the immune complex. The complement proteins will be consumed then the concentration of complement was reported to decrease. These results can shed light on the role of these proteins in various phases of the disease and could provide a basis for further exploration of the pathophysiological significance and suggest them for specific interventions.

Authors' Contribution

Conceptualization: AGa and AGh; Methodology: NK and SR; Formal analysis and investigation: GM and AGa; Writing the original draft: AGh and NK; Writing, reviewing, and editing: AGa; Supervision: AGa.

Availability of Data and Materials

Not applicable.

Competing Interests

The authors report no conflicts of interest.

Ethics Approval

All of the participants were informed about the objectives of the research and completed the consent forms. This study was approved by the Human Ethics Committee of Arak University of Medical Sciences, Arak, Iran (No IR.ARAKMU.REC.1399.079).

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