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ORIGINAL RESEARCH

Elevated levels of C3, C4, and CH50 of the complement system in ICU and non-ICU patients with COVID-19

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Abstract

Purpose: SARS-CoV-2 infection has spread in each corner of the world. Many health systems have dealt with it intensively. The complement system is an instrumental component in the inflammatory immune response and plays a role in the activation of blood coagulation. Our understanding of the pathophysiology of SARS-CoV-2 is still limited but is constantly expanding. This study aimed to determine changes in the complement system in intensive care unit (ICU) and non-ICU patients with COVID-19.

Methods: In a cross-sectional study, plasma levels of C3, C4, and CH50 were determined in two groups of ICU and non-ICU patients with COVID-19 to understand the potential effects of SARS-CoV-2 on the innate immune system. The assays of C3 and C4 were conducted using turbidimetry method. The CH50 test was conducted using the functional method.

Results: The present study revealed that the C3, C4, and CH50 plasma levels were 142.48 ± 30.38 mg/dL, 32.58 ± 8.78 mg/dL, and 61.74 ± 19.54%, respectively. These results indicate high levels of complement components C3 and C4 and complement function (CH50) in patients with COVID-19 than normal ranges. Plasma levels of C3, C4, and CH50 were higher in ICU patients than in non-ICU COVID-19 groups.

Conclusion: These results indicate that the innate immune system was activated in both ICU and non-ICU patients in response to SARS-CoV-2 infection. Further studies with a larger number of COVID-19 patients and additional testing of complement components (C3a and C5a) may reveal the role of COVID-19 infection in the activation of the complement system.

KEYWORDS
C3, C5, CH50, complement system, COVID-19, SARS-CoV-2

1 | INTRODUCTION

In March 2020, the World Health Organization (WHO) declared a COVID-19 pandemic.[1] According to WHO, 259502031 people have been definitively infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as of November 26, 2021. In addition, more than 5 183 003 deaths have been recorded due to COVID-19.[2] COVID-19 spreads 1000 times faster in the body than most related viruses.[3] Mild-to-moderate symptoms are predicted in the majority of COVID-19 patients. COVID-19 causes multiple organ dysfunction or acute respiratory distress syndrome in approximately 5% of cases.[4,5] In addition, symptoms in children are usually milder than in adults. Pneumonia is a more serious condition in adults than in children.[6] Multiple organ dysfunction in COVID-19 has been associated with a high risk of death due to advanced respiratory failure and systemic coagulopathy.[7,8]

The complement system, a core component of innate immunity, elicits proinflammatory responses during viral infections.[9] It has been hypothesized that activation of the complement system begins with direct lectin activation, followed by immune complex activation in the classical pathway, and finally by activation of the Toll-like receptor by the alternative pathway.[10] Activation of the C3 component of the complement system exacerbates acute respiratory distress syndrome, according to a report on SARS-CoV, which is closely related to SARS-CoV-2.[11] In five COVID-19 patients, skin and lung biopsies revealed deposits of C4d and C5b-9 in both skin and lung tissues. In addition, one patient with COVID-19 had an increased number of activated plasma cells in his bronchoalveolar lavage.[12] The relationship between complement activation and significant thrombophilia has been demonstrated previously.[13-15] In addition, activation of the coagulation system after inflammation has been demonstrated to be caused by downregulation of physiological anticoagulants, tissue factor-mediated thrombin generation, and inhibition of fibrinolysis.[16] Recent studies emphasize the prognostic value of D-dimer in COVID-19 infections.[17] The role of microvascular damage caused by complement activation and subsequent procoagulant conditions in the pathogenesis of SARS-CoV-2 in tissues is supported by these findings.[18] There is an urgent need to shed more light on this topic to understand the role of the complement system in the pathophysiology of COVID-19. In patients with COVID-19, the deposition of C4d and C5b-9 could be an indicator of strong activation of the alternative and lectin-based pathways of the complement system. If the disruptive role of the complement system in the deterioration of COVID-19 is confirmed, scientists may consider the use of complement inhibitors as a potential therapeutic option in patients with CIVID-19. Here, a cross-sectional study was conducted to examine C3, C4, and CH50 levels in two groups of patients with COVID-19 admitted to the intensive care unit (ICU) and outside the ICU. It is assumed that patients with COVID-19 admitted to the ICU completely are affected by SARS-CoV-2. Therefore, here, we tried to compare them in two groups of patients hospitalized for COVID-19.

2 | PATIENTS AND METHODS

2.1 | Patient population

Sixty patients with COVID-19 were enrolled in this pilot study from June to September 2020. The samples were collected from Ghaem and Imam Reza hospitals in Mashhad, Iran. Patients with COVID-19 who were eligible to participate in the study had to be at least 18 years old, have a documented COVID-19 infection in their medical records, have no concomitant diseases with COVID-19 such as cancer, hyperthyroidism, or rheumatoid arthritis, have no history of chronic hepatitis, cirrhosis, or liver dysfunction, and be taking no anti-inflammatory medications. An internist rigorously reviewed all medical histories, laboratory findings, and clinical presentations of the patients with COVID-19. After confirming that no comorbidities (other than hypertension) were present, patients were enrolled in the study. Under these circumstances, merely the influence of COVID-19 on the complement system would be measurable.

Exclusion criteria included:

1. Infestation with any of the above diseases after entering into the study.
2. Refusal to participate in the study.

The 60 patients with COVID-19 were divided into two groups: ICU patients and non-ICU patients with COVID-19 (30 patients in each group). The non-ICU patients with COVID-19 were patients who did not require admission to the ICU and did not require supplemental oxygen. These patients were selected from a general acute respiratory care unit.

Admission to an ICU and the need for intubation or ventilation were the prerequisites for admission in the intensive care category.

3 | LABORATORIES INVESTIGATIONS

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Four milliliters of whole blood were collected from the patients after the experiment was approved by the regional ethics committee of Mashhad University of Medical Sciences (approval number: IR.MUMS.REC.1399.153). To avoid transmission of SARS-CoV-2 to medical personnel, the regional ethics committee of Mashhad University of Medical Sciences approved the collection of blood samples from COVID-19 patients who were not awake without signing an informed consent form. The non-ICU patients with COVID-19 were informed about the research study. After the legal representatives of the participating patients gave informed consent, the required blood samples were collected. For the performance of the upcoming laboratory tests, the obtained sera were aliquoted and frozen after centrifugation at 1500*g* for 10 min.

4 | CH50

The CH50 assay was performed for all patients in one run after thawing the serum samples. The CH50 assay was conducted using the functional power of the complement system to lyse 50% of sheep red blood cells. The sheep red cells were pre-coated with rabbit antibodies against sheep red cells (hemolysin). The details of the procedure are described in the article by Dong and Liu.[19] The amboceptor used was purchased commercially from the Behring Company (Dade Behring, Marburg, Germany). A qualified laboratory technician experimented and read the results.

5 | C3 AND C4

Turbidimetry assay was used to determine C3 and C4 concentrations. C3 and C4 concentrations were measured using commercial biochemistry kits from Bionik Company (Tehran, Iran). Data were read using a Diatron instrument (Pictus 700). Normal values for C3 and C4 in adults were 90 to 180 and 10 to 40 mg/dL, respectively. All methods were performed in accordance with current guidelines and regulations. D-dimer, taken from medical records, was determined using commercial biochemistry kits from Bionik Company (Tehran, Iran).

6 | STATISTICAL METHODS

SPSS software (version 22) was used for statistical analysis. The Mann-Whitney test and the independent samples T-test were used to investigate possible statistical differences between C3 and C4 values in two groups of ICU and non-ICU patients with COVID-19. The independent samples T-test allowed comparative discrimination of CH50 value in two groups of ICU and non-ICU patients with COVID-19. Fisher's exact test was used to compare the mean of D-dimer levels in two groups of ICU and non-ICU patients with COVID-19.

7 | RESULTS

Among the 60 patients with COVID-19, there were 29 females (48.4%) and 31 males (51.6%). The youngest affected patient who participated in the study was 20 years old, while the oldest was 103 years old. The mean age of the patients was 57.70 ± 19.79 years. Table 1 showed the minimum, maximum, and mean ± SD of some of the major laboratory findings in these patients, including C3, C4, and CH50 levels. Table 2 listed these findings separately for the two groups of ICU and non-ICU patients with COVID-19.

Overall, patients with COVID-19 had increased plasma levels of C3, C4, and CH50 than normal ranges (Figure 1). All three markers were elevated in both ICU and non-ICU patients with COVID-19. Moreover, complement system activity, which measured as CH50 was increased in ICU and non-ICU groups (Table 2). Although there was no statistical difference in plasma levels of C3, C4, and CH50 between ICU and non-ICU patients with COVID-19, the plasma levels of the current markers in two groups of ICU and non-ICU patients with COVID-19 were higher than normal ranges.

The minimum, maximum, and mean of D-dimer in all patients were 100, 10 000, and 2484.75 ± 2184.86 ng/mL, respectively. The mean level of D-dimer was higher in ICU patients with COVID-19 than non-ICU patients with COVID-19 (2606.33 ± 1722.32 vs 2317.58 ± 2728.60 ng/mL respectively). The levels of D-dimer were elevated in both groups without a statistical difference, but were higher than the normal range (0-500 ng/mL).

8 | DISCUSSION

The complement system consists of several proteins that can lyse cells after activation.[20] The CH50 assay measures the ability of complement components to bind antibody-sensitized sheep erythrocytes. In other words, the CH50 assay is a screening tool to determine whether or not the classical pathway of the complement system has been activated. This process is mediated by assembling a membrane attack complex. In the case of SARS-CoV-2 infection, it is assumed that the cells infected by the virus are recognized by the body's immune

TABLE 1 The some important laboratory findings in 60 patients with COVID-19 (ICU and non-ICU)

Parameters	Minimum	Maximum	Mean ± SD	Normal range
WBC ($\times 10^9/\mu L$)	1.40	235	57.70 ± 30.26	4×10^9–$1.1 \times 10^{10}/\mu L$
Lymph (%)	3.7	63	14 ± 11.60	20–40
CRP (mg/L)	0.5	2813	739.05 ± 438	<6
ESR (mm/h)	2	130	55.47 ± 33.68	Up to 10
LDH (U/L)	287	9447	1074.37 ± 1374	Up to 440
O_2 sat (%)	51.70	99.70	72.84 ± 1.50	>97
C3 (mg/dL)	43	179	182.48 ± 30.38	80–160
C4 (mg/dL)	3	40	52.58 ± 8.78	10–40
CH50 (%)	24	124	61.74 ± 19.54	50%

Abbreviations: ICU, intensive care unit; Lymph, lymphocyte, CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; O_2 sat, oxygen saturation; WBC, white blood cell.

TABLE 2 Some major laboratory finding in two groups of the ICU and non-ICU patients with COVID-19 infection

	Minimum		Maximum		Mean ± SD	
Parameters	Non-ICU	ICU	Non-ICU	ICU	Non-ICU	ICU
WBC ($\times 10^3/\mu L$)	1.4	5.50	103	21.20	20.24 ± 42.34	9.91 ± 4.07
Lymph (%)	4	3.70	63	31.80	17.07 ± 14.86	11.04 ± 6.15
CRP (mg/L)	4	0.5	83.2	2810	35.44 ± 22.93	2254.51 ± 776.43
ESR (mm/h)	2	13	130	103	58.56 ± 41.06	52.13 ± 23.70
LDH (U/L)	287	437	2023	9447	658.16 ± 394.89	1497.44 ± 1873.42
O_2 sat (%)	55.20	98.0	53.4	99.70	73.88 ± 14.96	71.29 ± 17.30
C3 (mg/dL)	43	97	179	188	133.86 ± 34.10	151.10 ± 23.73
C4 (mg/dL)	5	3	40	48	32.20 ± 9.98	32.96 ± 7.54
CH50 (U/mL)	26	24	85	124	57.20 ± 15.26	66.27 ± 22.34

Abbreviations: ICU, intensive care unit; Lymph, lymphocyte, CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; O_2 sat, oxygen saturation; WBC, white blood cell.

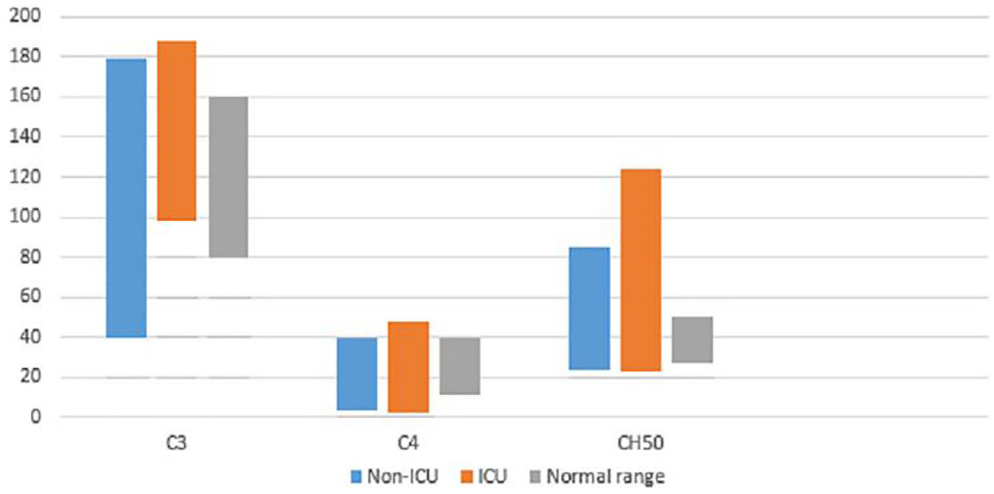

FIGURE 1 Comparison of C3, C4, and CH50 in ICU and non-ICU patients with COVID-19 groups with the relevant normal ranges. ICU, intensive care unit

system. The complement system would respond to the virus and the infected cells. Therefore, it is understandable that the plasma of infected patients would contain elevated levels of components of the complement system.

The current cross-sectional study showed that plasma levels of C3, C4, and CH50 are increased in both ICU and non-ICU patients with COVID-19. Moreover, the plasma levels of the above complement parameters are higher in ICU patients than in non-ICU patients with COVID-19. The plasma levels of C3 and C4 were higher in the ICU group than in the non-ICU group. This increase in plasma levels of C3 and C4 seems to parallel with the severity of COVID-19 and may indicate a trend between the severity of COVID-19 and levels of complement factors in plasma. Therefore, if larger groups are recruited in future studies, the difference could be statistically significant. Activation of the innate immune system in response to SARS-CoV-2 infection is evident in both ICU and non-ICU patients. Considering that the complement system promotes inflammation, elevated levels of C3, C4, and CH50 could be indicative of the role of the complement system in cytokine storm irritation in COVID-19.

There are limited data on the levels of C3 and C4 in COVID-19. Laurence et al.[21] reported elevated C3 levels in a patient with COVID-19 and found that anti-C5 treatment with Eculizumab produced the best results. There is increasing evidence of successful C3 inhibitor therapy in COVID-19 patients.[22,23] On the other hand, two papers reported normal C3 and C4 levels in patients with COVID-19.[24,25]

Compared with the standard C3 and C4 assays, the C3a and C5a assays will depict complement-driven disease activation.[26] Given that C3 and C4 levels may be insufficient markers of complement system activation in both ICU and non-ICU patients with COVID-19, it is recommended that C3a and C5a levels be measured in COVID-19. Furthermore, analysis of C3a and C5a levels in COVID-19 categories (mild, moderate, severe, and critical status) may provide additional insight. To cover the gaps, further studies are needed to shed light on the possible activation of complement pathways (classic, alternative, and lectin pathways) in SARS-CoV-2 infection and various infection severities. Besides this, the plasma levels of complement factors can be measured in comparison with interleukin 6 levels as a parameter that shows cytokine storm in patients with COVID-19.

To obtain clearer and more meaningful results, a study with a high number of patients with asymptomatic and ICU groups with COVID-19 is proposed. As we had financial constraints, this study was conducted on 60 patients with COVID19. Therefore, it is proposed to perform similar studies on a larger number of COVID-19 patients. In addition, studies on the other complement components would be beneficial to gain a better understanding of the activation of the complement pathway. There is still confusion about how long complement consumption should be measured, and which complement components should be measured.

9 | CONCLUSION

The pathogenesis of SARS-CoV-2 infection can be better understood if the details of complement activation in COVID-19 infection and various severities of infection can be elucidated. Inhibitors of the complement system may play a key role in the downregulation of cytokine storm in COVID-19. Hence, this suggestion may provide new therapeutics for cytokine storm management, thrombosis prevention, and other aspects of COVID-19 infection. As mentioned earlier, this was a pilot study to better understand the pathogenesis of COVID-19. It is expected that many more studies will uncover the mysteries behind SARS-CoV-2 infection.

ETHICS APPROVAL

This study was approved by The Regional Ethics Committee in Mashhad University of Medical Sciences (IR.MUMS.REC.1399.153).

STUDY APPROVAL STATEMENT

This study was approved by The Vice Chancellor of the Research in Mashhad University of Medical Sciences (Approval number 990243).

CONSENT FOR PUBLICATION

The regional Ethics Committee in Mashhad University of Medical Sciences issued an approval letter for this manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest regarding this manuscript.

AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Investigation: Writing—review and editing: Ahmad Bagherimoghaddam

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TRANSPARENCY STATEMENT

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The raw data are available on request from the corresponding author.

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