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ASSOCIATIONS BETWEEN SERUM LEVELS OF C3, C4, AND TOTAL CLASSICAL COMPLEMENT ACTIVITY IN COVID-19 PATIENTS AT THE TIME OF ADMISSION AND CLINICAL OUTCOME

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АССОЦИАЦИИ МЕЖДУ УРОВНЯМИ СЫВОРОТОЧНЫХ C3, C4 И ОБЩЕЙ АКТИВНОСТЬЮ КЛАССИЧЕСКОГО ПУТИ АКТИВАЦИИ

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КОМПЛЕМЕНТА У ПАЦИЕНТОВ С COVID-19 ПРИ ГОСПИТАЛИЗАЦИИ И КЛИНИЧЕСКИМИ РЕЗУЛЬТАТАМИ

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Abstract

In the present study, we investigated the association between complement system status at the time of admission and clinical outcomes in COVID-19 patients. This single-center study was carried out with sixty-one adult patients with COVID-19 who were hospitalized at Imam Hassan Hospital of North Khorasan University of Medical Sciences (Bojnurd, Iran) with less than three days passage since onset of COVID-19 symptoms. Twenty-three healthy volunteers with demographic features similar to the patient group (matched by age and gender) were included in the study as a control group. Patient information including demographic information, demographic data, clinical characteristics, and clinical outcomes were obtained from electronic medical records. Of 61 hospitalized patients with COVID-19, 28 (47.54%) were female, and the average age was 48.7 ± 8.8 years. The healthy control group included 23 cases (11 (47.8%) female, 12 (52.1%) males, mean age 46.4 ± 4.4 years). Twenty-one of the 61 patients (34.4%) were admitted to the ICU, and sixteen of them (26.2%) died. Thirty-three (54.10%) patients with COVID-19 were hospitalized for less than 7 days, and 28 (45.90%) of them were hospitalized for ≥7 days. Our results show that length of hospital stay in the no-ICU group was significantly lower than the ICU admission or death groups (6.49 ± 0.24 vs. 8.85 ± 1.59 and 10.53 ± 1.80, p =0.0002). The levels of C3, C4, and CH50 were determined through the immunoturbidimetric method and single-radial-haemolysis plates, respectively, on serum samples obtained from patients at the time of admission or those in the control group. Our results indicate that C3, C4 and CH50 levels were markedly lower in COVID-19 patients than in the control group. We also found that complement parameter levels in COVID-19 patients who died or were admitted to ICU were significantly lower than in non-ICU COVID-19 patients. In general, it seems that serum level of C3, C4, and CH50 at admission may predict disease progression or adverse clinical outcome in COVID-19 patients.

Keywords: COVID-19; complement system; clinical outcome; intensive care unit; discharge; mortality

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Резюме:

В настоящем исследовании мы оценивали связь между состоянием системы комплемента на момент поступления и клиническими исходами у пациентов с COVID-19. Одноцентровое исследование было проведено на 61 взрослом пациенте с COVID-19, которые были госпитализированы в больницу имама Хасана Северо-Хорасанского университета медицинских наук (Боджнурд, Иран), у которых с момента появления симптомов COVID-19 прошло менее трех дней. \В качестве контрольной группы в исследование были включены 23 здоровых добровольца, демографические данные которых были сходны с таковыми в группе пациентов (совпадали по возрасту и полу). Информация о пациентах, включая демографические данные, клинические характеристики и результаты, были получены из электронных медицинских карт. Из 61 госпитализированного пациента с COVID-19 28 (47,54%) были женщинами, а средний возраст составил 48,7 ± 8,8 года. В здоровую контрольную группу вошли 23 пациента (11 (47,8%) женщин и 12 (52,1%) мужчин, средний возраст 46,4 ± 4,4 года). Двадцать один из 61 пациента (34,4%) был госпитализирован в отделение интенсивной терапии (ОИТ), из них шестнадцать (26,2%) умерли, которые были госпитализированы в течение ≥7 дней начала заболевания. Наши результаты показали, что продолжительность пребывания в стационаре в группе без ОИТ была значительно ниже, чем в группах госпитализации и смерти в ОИТ (6,49 ± 0,24 против 8,85 ± 1,59 и 10,53 ± 1,80, P = 0,0002). Уровни C3, C4 и CH50 определяли иммунотурбидиметрическим методом и одномерным радиальным гемолизом соответственно в образцах сыворотки, полученных от пациентов на момент поступления и контрольной группы. Наши результаты показывают, что уровни C3, C4 и CH50 были существенно ниже у пациентов с COVID-19, чем в контрольной группе. Мы также обнаружили, что уровни этих значений параметров комплемента у пациентов с COVID-19, умерших или поступивших в ОИТ, достоверно ниже, чем у пациентов с COVID-19, не поступивших в отделение интенсивной терапии. В целом можно полагать, что уровень сывороточных C3, C4 и CH50 при поступлении может предсказать прогрессирование заболевания и неблагоприятные клинические исходы у пациентов с COVID-19.

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Ключевые слова: COVID-19; система комплемента; клинический результат; отделение интенсивной терапии; выписка; смертность

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Introduction

More than a year after its emergence, COVID-19, the disease caused by SARS-CoV-2, continues to plague the world and dominate our daily lives (2). Patients with COVID-19 may develop symptoms such as fever, dry cough, pharyngeal pain, tiredness, abdominal pain, diarrhea, conjunctivitis, muscle fatigue, or pneumonia; some are left with serious side effects or even die (14). Previous studies have reported that 14.1–33.0% of COVID-19 patients are prone to develop into severe cases, and the mortality rate of critical cases is 61.5%, increasing sharply with age and underlying comorbidities (23, 22). In more severe cases of COVID-19, patients can develop acute respiratory distress syndrome (ARDS), leading to a worse prognosis (9). Deregulated activation of multiple adaptive and innate immune pathways (including T cell activation, cytokine expression from macrophages and neutrophils, the complement system, and several procoagulant and thrombogenic pathways) is believed to fuel a hyperinflammatory state that drives ARDS and may lead to multiple organ injury and finally death in COVID-19 (13, 19).

The complement system is a key part of the innate immune system which plays an important role in defense against foreign pathogens such as viruses but, in addition to being an important part of the immune defense system, it plays a critical role in promoting the inflammatory process that leads to organ dysfunction (21). Although several studies have been carried out on the complement system in COVID-19 and its relationship with clinical outcomes (5, 8, 6, 12, 25), they did not indicate a clear protective or adverse effect of this system. Dheir*et al*. in a retrospective study showed there is no significant difference, in terms of C3 and C4 levels, in both ICU and non-ICU COVID-19 patients (5). They suggested that measurement of C3 and C4 levels cannot be used to show severity disease (5).

Controversially, Ghazavi et al. found that the levels of C3 and C4 in non-severe COVID-19 patients were significantly higher than in severe-COVID-19 patients (8). A previous study by Fang *et al.* also indicated decreased complement C3 levels are associated with poor prognosis in COVID-19 patients (6). Java and colleagues claimed that the role of the complement system in COVID-19 patients is time dependent, wherein complement activation in the first week of infection can serve as a “friend”, and its activation in the second or third weeks of infection can be a “foe” (12). Zinellu *et al*., in a systematic review, concluded that lower

concentrations of C3 and C4 are significantly associated with higher COVID-19 severity and mortality (25). They suggested that additional studies are required to determine whether measurement of complement components can be useful to predict adverse clinical consequences in COVID-19 patients (25).

Therefore, we conducted a study to assess the association between complement system status at the time of admission and clinical outcomes (e.g., length of stay, ICU admission, discharge, mortality) in COVID-19 patients. Understanding this association will help us elucidate the role of the complement system for prediction of the risk of developing critical COVID-19.

Materials and methods

Study design and participants

This single-center study was carried out with sixty-one adult patients with COVID-19 who were hospitalized at *Imam* Hassan Hospital of North Khorasan University *of medical sciences* (Bojnourd, Iran) from April 20 to August 5, 2021 with less than three days passage since the onset of COVID-19 symptoms. All patients with a positive RT-PCR test for SARS-CoV-2 and common CT imaging findings associated with COVID-19 were included in the study. Patients also had no history of SARS-CoV-2 infection or COVID-19 vaccination.

Available data suggest that patients with mild-to-moderate COVID-19 remain infectious no longer than three days after symptom onset. Recent evidence indicates that age and comorbidities could possibly confound the association between complement system status and clinical events (16). Therefore, we adjusted for sex, age and comorbidities. Twenty-three healthy volunteers with demographic features similar to the patient group (matched by age and gender) were included in the study as a control group.

Data collection and assessment of serum complement levels and activity

We obtained demographic data, exposure history, chronic medical histories, clinical symptoms or signs, clinical outcomes, and hospitalization duration from electronic medical records. We also collected serum samples from PCR-confirmed COVID-19 patient samples sent to Imam Hasan Hospital laboratory on the first day of admission for analysis of serum levels of C3, C4, and total classical complement

activity (CH50 assay) present in the serum. We also collected blood samples from the healthy controls with a negative PCR-test for COVID-19. Blood samples were centrifuged immediately, and serum was obtained and frozen at -70°C until use.

Serum C3 and C4 concentrations were determined using kits from Roche Diagnostics (Indianapolis, IN, USA) according to the immunoturbidimetric method (18). The CH50 test was evaluated using single-radial-haemolysis plates (Biogen, Iran) as described elsewhere (18). All values were compared to the normal ranges which were reported as: 89-187 mg/dL for C3; 10-40 mg/dL for C4; and70-130 mm for CH50.

Statistical analysis

All statistical analyses were carried out using GraphPad Prism 5.0 (GraphPad, San Diego, CA, USA). Data distribution was analyzed by a Kolmogorov-Smirnov test. According to the results of the normality test, a one-way ANOVA followed by Dunn's or Tukey's post-hoc test, or a non-parametric Kruskal-Wallis test, were used for statistical comparisons. Analyses were adjusted for age, sex and comorbidities. Data were expressed as mean ± standard deviation (SD). Values of $p < 0.05$ (*) were considered significant.

Results

Demographic data and clinical characteristics

Of 61 hospitalized patients with COVID-19, 28 (47.54%) were female, and the average age was 48.7 ± 8.8 years. The most commonly self-reported symptoms at onset of illness were fever (n = 41 [67.2%]), cough (n = 39 [63.9%]), fatigue or myalgia (n = 19 [31.4%]), diarrhea (n = 9 [14.7%]), or headache (n = 6 [9.8%]). Twenty-six (42.6%) patients had comorbidities, including cardiovascular disease (n = 10 [38.4%]), diabetes (n = 6 [23.0%]), hypertension (n = 7 [26.9%]), chronic kidney disease (n = 3 [11.5%]), and endocrine system diseases (n = 2 [7.6%]). The healthy control group included 23 cases (11 (47.8%) female, 12 (52.1%) males, mean age 46.4 ± 4.4 years).

Twenty-one of the 61 patients (34.4%) were admitted to the ICU, and sixteen of them (26.2%) died. Thirty-three (54.10%) patients with COVID-19 were hospitalized for less than 7 days, and 28 (45.90%) of them were hospitalized for ≥7

days. Our results show that length of hospital stay in the non-ICU group was significantly lower than in the ICU admission or death groups (6.49 ± 0.24 vs. 8.85 ± 1.59 and 10.53 ± 1.80, $p = 0.0002$).

C3, C4, and CH50 levels in COVID-19 patients and the healthy control group

As shown in Table 1, serum C3 and C4 concentrations and CH50 assay were markedly lower in COVID-19 patients than in the healthy control group. Further statistical analysis showed that serum levels of C3, C4, and CH50 in the non-ICU admission group were statistically higher than in the death and ICU admission groups. We did not observe a significant difference in C3, C4, and CH50 levels between ICU admission and death groups of COVID-19 patients ($p > 0.05$).

We also analyzed the relationship between C3, C4, and CH50 levels in COVID-19 patients and hospital length of stay. As shown in Figure 1, serum levels of C3, C4, and CH50 at the time of admission in COVID-19 patients who were hospitalized for 7 or more days were statistically lower than in COVID-19 patients who were hospitalized less than 7 days ($p < 0.05$).

Discussion

The complement system efficiently recognizes and eliminates viral pathogens via several mechanisms including: opsonization of viruses; lysing of virus-infected cells; induction of an antiviral immunoinflammatory state; boosting of virus-specific immune responses; and directly neutralizing cell-free viruses (1). On the other hand, complement activation in viral infections may play a critical role in pathogenesis, clinical manifestation, and disease severity (3, 10).

Several reports have shown that the concentration of complement components, and serum complement activity, change in the COVID-19 patients. However, they did not definitively determine whether measurement of complement components or serum complement activity can be useful to predict adverse clinical outcomes in COVID-19 patients. Therefore, this study was designed to assess the relationship between serum levels of C3, C4, and total classical complement activity in COVID-19 patients at the time of admission and clinical outcomes. The results of this study indicate that serum levels of C3 and C4 and CH50 assay were markedly lower in COVID-19 patients than in the healthy control group. The most interesting

finding was that serum levels of C3, C4, and total classical complement activity in COVID-19 patients who died or were ICU admitted were significantly lower than in COVID-19 patients who were not ICU admitted.

Conversely, Keshavarz and colleagues showed that serum levels of the C3 and C4 factors have no significant change between patients and healthy individuals (15). In another study, Zhang et al. showed that complement C3 cannot predict disease progression (24). Our findings are also contrary to that of Dheirs *et al*. who found there was no significant difference in terms of C3 and C4 levels in both ICU and non-ICU COVID-19 patients (5). Henry et al. have also shown that complement hyperactivation failed to predict progression to severe COVID-19 (11). In addition, they also showed that there are no significant differences in total classical complement activity (or CH50 level) at the time of admission between COVID-19 patients with different clinical outcomes (11). These inconsistencies may be due to differences in study design, limited sample size, characteristics of COVID-19 patients, time between the onset of symptoms and obtaining the blood sample, or confounders.

However, this study supports the findings from previous observations (6, 8, 15). Fang et al., in a retrospective cohort study, investigated C3 levels in COVID-19 patients (6). They showed that C3 levels in the non-survival group were significantly lower than in the survival group; they concluded that decreased complement C3 levels are associated with poor prognosis in COVID-19 patients (6). The finding also match earlier findings by Ghazavi *et al.*, which showed that C3 and C4 levels were markedly decreased in severe COVID-19 patients in comparison with non-severe COVID-19 patients (8). In agreement with our data, Keshavarz *et al*. indicated that mean CH50 activity level in COVID-19 patients is significantly reduced compared to healthy individuals (15).

The decreased levels of C3 and C4 in COVID-19 patients (in comparison to the healthy group), and significant differences between the non-ICU admission group and the death and ICU admission groups, may be explained by the fact that hyperactivation of the complement system leads to proteolytic cleavage of the key complement molecules C3and C4, leading to cleavage products including C3a, C3b, C4a, and C4b. These may trigger severe inflammatory responses in numerous organs (4). In support of this possibility, Brandon and colleagues have demonstrated that

C3a and the C3a/C3 ratio are significantly elevated in severe COVID-19 patients presenting to the emergency department compared to mild or moderate severity COVID-19 patients (11). On the other hand, Fletcher-Sandersjöö and colleagues suggested that overactivation of the complement cascade in COVID-19 patients is associated with activation of coagulation systems and consequent severe complications (7). It is possible, therefore, that overproduction of C3a and C4a following complement hyperactivation is associated with adverse outcomes in patients with SARS-CoV-2 infection through activation of the coagulation cascade and thrombus formation.

Taken together, these results suggest that the levels of complement C3, C4, and CH50 at admission may predict disease progression and adverse clinical outcome in patients with SARS-CoV-2 infection. These findings may be somewhat limited by several confounders, such as different viral strains, sociodemographic factors, and various types of drug therapy during hospitalization (20, 17). Thus, we suggest that future studies include: investigation of alteration of C3 and C4 at different stages of the disease; determination of the relationship between serum levels of C3 and C4 and inflammatory biomarkers; and design of clinical trials with complement inhibitors, such as a C3 inhibitor.

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Declarations

Ethics approval

The study was performed based on an informed consent and was approved by the Ethics Committee of North Khorasan University of Medical Sciences, Bojnourd, Iran (IR.NKUMS.REC.1399.021).

Informed consent: Informed consent was obtained from all individual participants included in the study.

Conflict of interest: The authors declare that they have no conflict of interest.

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TABLES

Table 1. Comparison of serum C3, C4 and CH50 levels at the time of admission between COVID-19 patients with different clinical outcomes and the healthy control group.

Таблица 1. Сравнение сывороточных уровней C3, C4 и CH50 на момент госпитализации между пациентами с COVID-19 с разным клиническим исходом и здоровой контрольной группой.

Lab Variable Параметр	**Healthy Control Здоровый контроль**	**No-ICU Admission Без поступления в ОИТ**	**ICU Admission Поступление в ОИТ**	**Death Смерть**	***P* value *P* величина**
C3, mg/dl C3, мг/дл	137.1 ± 25.82	98.76 ± 19.09	72.62 ± 13.92	71.63 ± 14.28	<0.0001
C4, mg/dl C4, мг/дл	28.50 ± 6.25	24.46 ± 5.87	17.71 ± 6.96	18.79 ± 5.44	<0.0001
CH50, mm CH50, мм	94.43 ± 13.66	91.58 ± 17.89	77.24 ± 12.66	78.50 ± 7.64	0.007

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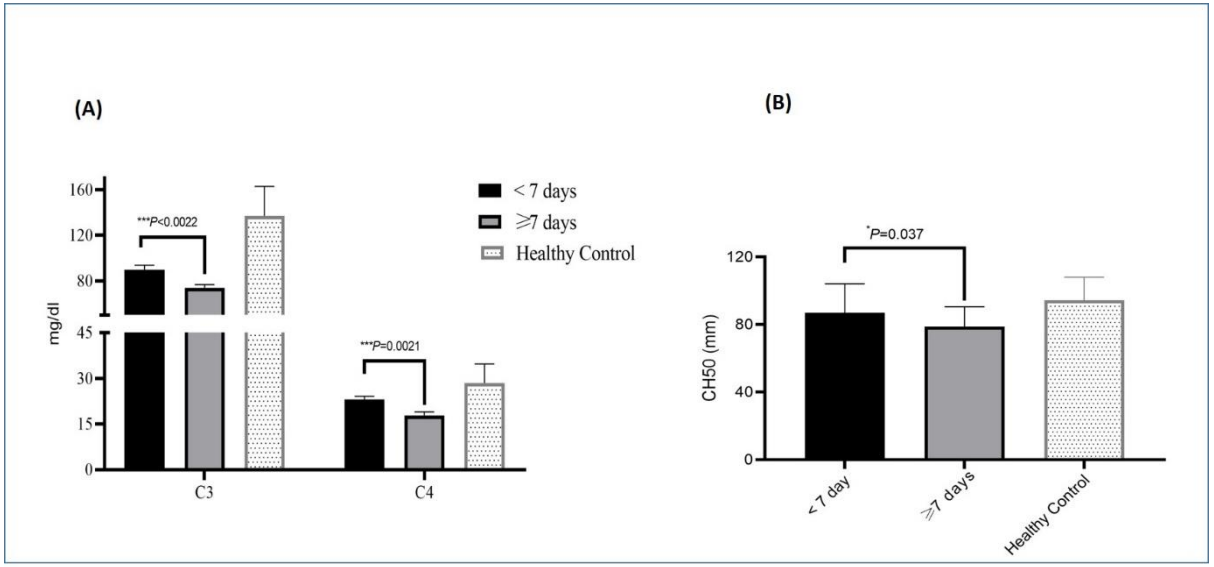
FIGURES

Figure 1. Comparison of hospital stay length in COVID-19 patients with serum levels of C3, C4 and CH50 at the time of admission. Data are mean ± SD. *$p < 0.05$ was considered statistically significant.

Рисунок 1. Сравнение продолжительности пребывания в стационаре у пациентов с COVID-19 с сывороточными уровнями C3, C4 и CH50 на момент поступления.

Данные представляют собой среднее значение ± стандартное отклонение. *P <0,05 считалось статистически значимым.



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АССОЦИАЦИИ МЕЖДУ УРОВНЯМИ СЫВОРОТОЧНЫХ C3, C4 И ОБЩЕЙ АКТИВНОСТЬЮ КЛАССИЧЕСКОГО ПУТИ АКТИВАЦИИ КОМПЛЕМЕНТА У ПАЦИЕНТОВ С COVID-19 ПРИ ГОСПИТАЛИЗАЦИИ И КЛИНИЧЕСКИМИ РЕЗУЛЬТАТАМИ

Running head: Complement system in COVID-19 patients
Сокращенное название: Система комплемента у пациентов с Covid-19

Keywords: COVID-19; complement system; clinical outcome; intensive care units; discharge; mortality.
Ключевые слова: COVID-19; Система комплемента; Клинический результат; отделения интенсивной терапии; Увольнять; Смертность.

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