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COMPLEMENT LEVEL CHANGES AMONG IRAQI COVID-19 PATIENTS

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Abstract

The complement system plays a significant role in the human innate immune system during the COVID-19 pandemic exhibits several protective effects against the virus in order to eliminate cellular debris, induced inflammation. On the other hand, it plays a significant role in the pathogenesis of COVID-19, primarily through its involvement in multi-organ dysfunction and increasing the likelihood of adverse clinical outcomes. In order to confirm that the complement system contributes to COVID-19 pathogenicity, it is essential to measure the concentration of complement system proteins in COVID-19 patient sera compared to healthy individuals. This study aims to evaluate and quantify the level of C1q, C3, C4, and Properdin between the patients diagnosed with COVID-19 and control groups, specifically focusing on the statistically significant variations in patients' outcomes compared to the non-significant changes. This study measured classical pathway activation markers (C1q, C3, C4, and Properdin) in 70 Iraqi patients with COVID-19 as well as 20 healthy controls. Levels were assessed by the ELISA technique in Medical City hospitals in Baghdad between December 2022 and May 2023. Patients infected with SARS-COV-2 reported decreased levels of (C1q, C3, and C4) in their blood when compared with controls, as shown in Figure 1. In addition, C1q, C3, and C4 levels showed a notable disparity between their levels in the sera of patients in comparison to the healthy control ($p=0.0164$), ($p=0.0282$), ($p=0.0454$) respectively, with no statistically significant difference seen in Properdin levels between the study groups ($p=0.8837$). Based on the available evidence, this work represents the initial investigation of C1q, C3, C4, and Properdin in COVID-19 patients in Iraq. In conclusion, according to the complement level in serum, there was a highly significant disparity between patients with COVID-19 and the control group, except for Properdin level. It might be due to the classical pathway's hyperactivation and complement protein consumption during infection.

Introduction

Severe acute respiratory syndrome (SARS) is a severely critical viral respiratory disease. The virus known as coronavirus 2 (SARS-CoV-2) is responsible for the onset of coronavirus disease 2019 (COVID-19). This infection develops a spectrum of severity, ranging from mild and moderate symptoms to severe pneumonia. In some instances, patients may have mechanical ventilation assistance, and unfortunately, fatalities can occur (Huang et al., 2020).

The complement system represents an important and crucial constituent of the host immune system. The system generally has a hepatic-originating and plasma-mediated function that continuously monitors the bloodstream for the presence of foreign pathogens and endogenous antigens. The pathogen induces only one or all complement activation routes, including the classical, lectin, or alternative pathways. This results in the enzymatic cleavage and subsequent activation of the essential components C3 and C5 by C3 and C5 convertases, respectively. As a result, bioactive molecules such as C3a, C3b, C5a, and C5b will be generated. (Noris and Remuzzi, 2013).

The activation of the complement system occurs through three distinct paths, including the classical, lectin, and alternative pathways. These pathways ultimately converge at the cleavage of a component known as complement component 3 (C3) (Merle et al., 2015).

The classical pathway activation mainly occurs through the interaction between C1q and immunological complexes containing IgG and IgM. On the other hand, the lectin pathway is triggered upon recognition of non-self-carbohydrate structures by mannan-binding lectin (MBL). Conversely, the alternate pathway is initiated through the spontaneous hydrolysis of C3, producing C3b. Subsequently, C3b interacts with a range of proteins, lipids, and carbohydrates present on the surface of the pathogen (Merle et al., 2015).

There is a hypothesis suggesting that natural IgA and IgM antibodies play a role in enhancing the lectin pathway (LP) by recruiting C1q. This augmentation of the LP facilitates complement activation and can contribute to the clearance of the virus. Severe disease manifestation and the possibility of a harmful complement response initiated by complement deficiency occur only when the virus exceeds the initial immune response and infiltrates the alveoli, which do not have mucosal complement-dependent protection, and when viral replication and a strong antibody response have been initiated (Matricardi et al., 2020).

Observations on circulating blood cells provide additional support for activating the systemic complement pathway in individuals with COVID-19. The monocytes exhibited a robust staining pattern for C1q. The observed deposition was correlated with an elevated presence of C3 (Lage et al., 2022). C1q has reported deposition in the pulmonary region. Additionally, other investigations have reported the deposition of C4 and C3(Satyam et al., 2021).

The study conducted by Wu et al. (2020) proposes that reduced plasma C1q levels may indicate mortality in individuals at high risk. It suggests the existence of a crucial balance between C1q levels and antibody levels. (Wu et al., 2020)

The work conducted by Castanha et al. provides evidence to support the association between CP

activation and the severity of COVID-19 disease and its role in triggering complement-mediated hyperinflammation. This study observed a notable elevation in plasma levels of C1q among individuals in the hospitalized group, which positively correlated with the disease severity. A positive correlation is found between the levels of IgG antibodies targeting the S- and N-proteins and the presence of IC-C1q. The findings of Castanha et al. (2022) indicate that this particular complement activation pathway marker is the primary contributor to the development of severe disease, as proven by comparisons with other markers.(Castanha et al., 2022)

The recent data analysis has revealed a notable decrease in circulatory levels of the properdin protein among those diagnosed with severe COVID-19. Conversely, there was a considerable upregulation in the expression of the properdin gene in these patients. (Boussier et al., 2022) The accumulation of Properdin in tissues may lead to decreased levels of Properdin in blood circulation.

Numerous investigations have examined the role and association of the complement system with COVID-19 clinical manifestations. However, this research has not yielded conclusive evidence about a definitive protective or detrimental effect produced by this system, and a retrospective study conducted by Dheir et al. observed the absence of a statistically significant distinction in C3 and C4 levels between patients with COVID-19 in intensive care units (ICUs) and those in non-ICU settings. (Dheir et al., 2020).

According to Dheir et al. (2020), it has been proposed that assessing C3 and C4 levels may not indicate illness severity. (Dheir et al., 2020) In a controversial manner, Ghazavi and colleagues discovered that non-severe COVID-19 individuals exhibited considerably elevated levels of C3 and C4 compared to patients with severe disease manifestations. (Ghazavi et al., 2020).

A prior investigation conducted by Fang et al. demonstrated a correlation between diminished levels of complement C3 and an unfavorable outcome among individuals affected by COVID-19 (Fang et al., 2020). According to Java findings, the complement system in individuals with COVID-19 depends upon the activation timing. They propose that complement activation during the first few days of infection may benefit, acting as a "friend." However, activation during the period from the second to the third weeks of infection may have a detrimental effect, acting as a "foe" (Java et al., 2020).

According to the findings of Zinellu et al. in their systematic review, a strong association exists between increased COVID-19 severity and mortality with a decreased level of C3 and C4 (Zinellu and Mangoni, 2021). The authors proposed that further investigations are necessary to investigate the potential use of measuring complement components in predicting unfavourable clinical outcomes among individuals with COVID-19 (Zinellu and Mangoni, 2021).

In this concise report, the study examines the alteration of the complement components of the classical pathway in the sera of persons who suffered from COVID-19. The study reports that components of the classical (C1q, C3, C4) level were lower in people with COVID-19, indicating the activation of the classical pathway and (Factor P) level with no significant change.

2. Materials and Methods

2.1. Study design

The study included seventy Iraqi patients aged (20-45) years with COVID-19 (42 male and 28 female) who attended Medical City Hospitals from December/2022 to March/2023 for diagnosis and receiving care and treatment. Twenty healthy persons aged (20-45) years from medical staff are chosen as a control group.

The blood samples were collected from collectively studied participants and utilized study objectives with a written informed approval form for sample collection. Exclusion criteria in order to enrol in this study were patients with a history of chronic disease, pregnant females, and patients with clinical or pathological evidence of cancer and autoimmune disease or who received any therapy.

Venous blood samples were collected from each subject in this study. Centrifugation was used to obtain serum, which was immediately stored at -20 C until testing. Serum levels of C1q, C3, C4, and Properdin were determined using an enzyme-linked immunoabsorbent assay (ELISA) technique (Sun Long/China) according to the manufacturer's protocol.

2.2. Statistical analysis

Statistical analysis was utilized for all data analysis using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA). The data are expressed as means ± standard deviation and median: the student's t-test and one-way ANOVA (Tukey's test) determined variables between study groups. Statistically, significance is defined by the significance levels expressed by p-values, commonly represented as * $p < 0.05$ and ** $p < 0.01$.

3. Results

This study found that the serum levels of C1q, C3, C4, and Properdin in the patient group are illustrated in Fig. 1. Determination of level C1q showed a significant difference between its level in the sera of patients (264.7 ± 148.2) pg/ml in comparison to the healthy control (366.9±214) pg/ml ($P = 0.0164$), as shown in Fig. 1-A. While C3 level in patients group was (22.91±15.24) ng/ml and control group were (33.22 ±26.33) ng/ml show significant difference ($p=0.0282$) illustrated in Fig (1-B) Furthermore, the C4 level in the patient group was (13.59±6.105) ng/ml compared to the control group (16.86± 6.823) ng/ml, with a significant difference ($p = 0.0454$) as

illustrated in Fig. 1-C.

Properdin in the patients group (357.5±160.2) ng/ml and control group (363.8 ± 198.7) ng/ml, but with a non-significant difference between the groups (p = 0.8837) as shown in Fig. 1D.

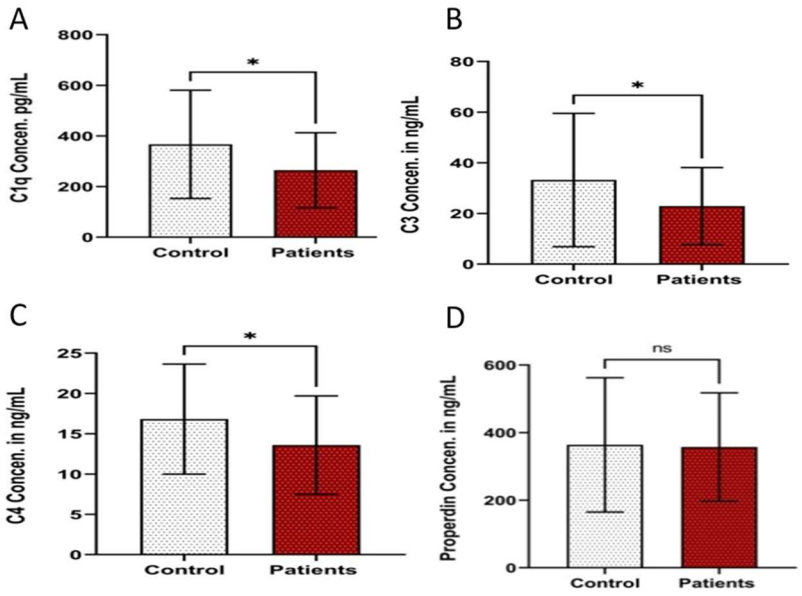

Figure 1: The level of the complement system is described as the mean and SD, as shown in figures 1–A. C1q pg/ml (1-B) C3 (ng/ml), (1-C) C4 (ng/ml), and (1-D) properdin (ng/ml) among the study groups Data analysis is conducted by applying the Student's *t*-test to determine whether the group variance is significant or not.

The associations between serum levels of complement classical pathway activation markers among patients with COVID-19 are listed in Figure (2).

A significant positive correlation was seen between C1q and C3 in patients (P = 0.0989), as shown in Figure (1-A).A highly significant correlation (P = < 0.0001) between C1q and Properdin can be seen in Figure (1-C), while a highly significant negative correlation was seen between C1q and C4 in the patient's (P = 0.0038) as shown in Figure (1-B). Figure (1-D) represents a highly significant positive correlation between C3 and C4 (P = 0.0001).

No significant correlation was seen between Properdin and C3 patients (P = 0.3801), as shown in Figure (1-E), while its correlation with C4 showed a highly significant negative correlation (P = 0.0001), as shown in Figure (1-F).

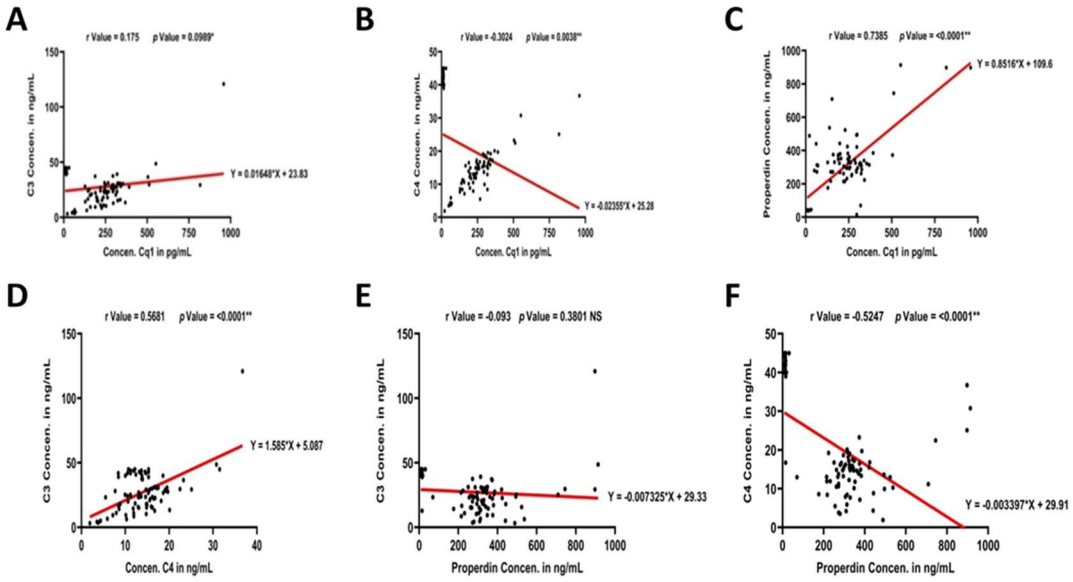

Figure (2) shows the correlation between the complement system of classical pathway activation markers. Figure (2-A): Simple Linear Regression between C1q(pg/ml) and C3(ng/ml) Figure (2-B): Simple Linear Regression between C1q(pg/ml) and C4(ng/ml) Figure (2-C): Simple Linear Regression between C1q(pg/ml) and Properdin (ng/ml) Figure (2-D): Simple Linear Regression between C4(pg/ml) and C3 (ng/ml) Figure (2-E): Simple Linear Regression between Properdin (pg/ml) and C3 (ng/ml) Figure (2-F): Simple Linear Regression between Properdin (pg/ml) and C4 (ng/ml) The Pearson coefficient r was employed to assess correlation. The significance levels expressed by p-values are commonly represented as * $p < 0.05$ and ** $p < 0.01$.

4. Discussion

The global pandemic caused by the SARS-CoV-2 virus has resulted in a significant number of fatalities on a global scale (Cascella et al., 2023). Several factors have been identified as contributing to a greater susceptibility to severe COVID-19, including age, genetic predisposition, and immune system disturbance (Antos et al., 2021). Therefore, acquiring an understanding of the pathogenicity of SARS-CoV-2 is essential for effectively managing the virus and preparing for future outbreaks.

The complement system is the key player that plays an important role in the innate immune response by marking pathogens, mediating lysis, and attracting inflammatory cells to the infection site (Polycarpou et al., 2020).

Extensive data has evolved throughout the pandemic, revealing the disruption of the complement system pathway and its role in the immune-pathological effects of COVID-19. This study finding approved the previous studies and investigations for the involvement of these system components.

COVID-19 may trigger activation of complement via different pathways, especially the classical and alternative pathways, causing an accumulation of opsonin molecules, such as C3b, in COVID-19 patients (Gao et al., 2022).

IgM autoantibodies that specifically target angiotensin-converting enzyme-2 (ACE2) on endothelial cells indicate a T-independent antibody response. (Casciola-Rosen et al., 2020) This immunological response triggers the complement pathway (CP) and induces an inflammatory reaction. (Magro et al., 2020)

It can be concluded that there is excessive activation of complement protein mediators in the classical path. Over time, the amounts of these proteins will decrease due to their consumption, as seen by measuring their levels in the serum of patients, which showed a decrease in C1q, C3, and C4 levels.

The previous study done by (Alosaimi et al., 2021) found that lower C2 and C1q levels in critical patients are believed to complement the depletion during activation, which approved our hypothesis.

Previously mentioned results that conducted the dropping in (C1q, C3, C4) level in agreement with multiple investigations that documented the activation of the complement system by SARS-CoV-2 through all three pathways. Moreover, the deregulation of the classical route has been linked to the pathogenesis observed in SARS-CoV-2 infection, as reported by Siggins (Siggins et al., 2022).

A recent investigation concerning SARS-CoV, a virus closely related to SARS-CoV-2, discovered that the activation of complement component C3 contributes to the worsening of disease in SARS-CoV-associated Acute Respiratory Distress Syndrome (ARDS). The study observed that mice infected with SARS-CoV deficient in C3 experienced reduced respiratory dysfunction. This improvement was linked to decreased infiltration of neutrophils and inflammatory monocytes in the lungs and lower levels of cytokines and chemokines in both the lungs and the bloodstream (Gralinski et al., 2018).

Several studies have shown that differences in complement system level are interpreted as significant predictions of severity and mortality in COVID-19. Risitano et al., . given that C3 activation is the convergence point or the key to the activation of all complement pathways (Sinkovits et al., 2021), study revealed that the population of non-survivors exhibited a significant reduction in C3 level, accompanied by a significant elevation in C3a/C3 ratios. Furthermore, the study revealed a significant drop in C4 levels within the non-survivors group, reflecting the existence of a robust correlation between the severity of COVID-19 and high activation and consumption, with a possibility of the lectin or classical pathways being involved. In addition, (Fang et al., 2020) reported significantly lower serum C3 levels in a retrospective cohort study. It was correlated with the mortality rate of COVID-19 and poor prognosis in the same study.

Decreased C3 levels, hypothesized due to over-activation and utilization, protein loss, or reduced production, have yet not to be fully understood; the study has not analyzed the complement activation markers.

Therefore, this study investigated the changes in (C1q, C3, C4, and Properdin) levels during SARS-CoV-2 infection and observed a decrease in classical complement activation markers, as shown in Figure 1; C1q, C3, and C4 levels in the patient group were lower than in the control group, which may be due to the complement system activation.

Zhang's study revealed that complement C3 cannot be identified as a reliable predictor for indicated disease progression (Zhang et al., 2020). Henry also showed that hyperactivation of complement failed to indicate the progression to severe COVID-19. Furthermore, the study revealed no statistically significant disparities in the overall classical complement activity, also known as CH50 level, among patients diagnosed with COVID-19 upon entry to the hospital. (Henry et al., 2021)

Complement C4, a key molecule in the complement system that is one of the chief constituents of innate immunity for the immediate recognition and elimination of invading microbes, plays an essential role in the functions of both the classical (CP) and lectin (LP) complement pathways. (Zinellu and Mangoni, 2021)

The C4 protein plays a crucial role in the immune response through both the classic and lectin complement pathways. The previous research demonstrated the neutralization of complement by encapsulated viruses through the action of C4.

The reduction in C4 levels in COVID-19 patients in association with COVID-19 severity was supported by several studies, such as (Yahya et al., 2023) and (Ghazavi et al., 2020) in Iran, which found that complement C3 C4 levels were markedly reduced in COVID-19 patients affected by serious conditions in comparison with those without severe disease.

Al-Hakeim et al. study conducted in Iraq also discovered that patients who remained in the hospital for more than 15 days exhibited decreased levels of complement C4 compared to individuals who left the hospital earlier (Al-Hakeim et al., 2021). As shown in figure (1 C) decreased C4 levels decreased when comparing infected individuals with COVID-19 and non-infected individuals. The interactions of viruses, bacteria, and some pathological conditions cause the consumption or inhibition of complement C4 through the classic or lectin complement pathway (Zinellu and Mangoni, 2021).

Multiple studies have indicated alterations in the levels of complement components and serum complement activation factors in individuals affected by COVID-19. (Razi et al., 2023) A study has been conducted that exhibits a significant decrease in serum levels of C3 and C4, as well as

the CH50 test, in patients diagnosed with COVID-19 compared to a control group of healthy individuals. The most important discovery in the previously mentioned study was the reduced levels of C3, C4, and overall classical complement activity observed in COVID-19 patients who were either admitted to the intensive care unit (ICU) or deceased, compared to those who were not admitted to the ICU. Individuals diagnosed with COVID-19.

In contrast, when comparing patients to healthy persons, Keshavarz et al. demonstrated no statistically significant alteration in the serum levels of the C3 and C4 factors (Keshavarz et al., 2021). The study observations contradict those of Dheirs, who reported no statistically significant disparity in C3 and C4 levels between patients admitted to the intensive care unit (ICU) and those not admitted to the ICU and individuals diagnosed with COVID-19 (Dheir et al., 2020).

While Fletcher-Sandersjöö et al. proposed that excessive activation of the complement cascade in individuals with COVID-19 is linked to the activation of coagulation systems, resulting in severe problems, (Fletcher-Sandersjöö and Bellander, 2020).

As mentioned previously, the complement system is specifically intended to identify and destroy invading pathogens by activating the classical, alternative, and lectin pathways. Human properdin enhances the stability of the alternative pathway C3 convertase, creating a positive loop that promotes the synthesis of C5 convertase. As a result, it functions as a positive regulator of the alternative pathway. (Varghese et al., 2021)

Properdin, the positive AP regulator, is essential for complement amplification by stabilizing enzymatic convertases (Chen et al., 2020) . Boussier et al. reported that levels of Properdin, a positive regulator factor of the alternative pathway, were markedly reduced in severe COVID-19 patients compared to moderate patients and associated with COVID-19 complications as requiring mechanical ventilation, demonstrating its predictive value for disease outcome (Boussier et al., 2022).

Properdin levels remained low in severe COVID-19 across the disease course. Properdin stabilizes the alternative pathway convertase; reduced plasma levels would thus be anticipated to reduce alternative pathway activation. (Chen et al., 2018) In our study, properdin level does not change the probability that the classical pathway is the main path that the virus takes. Or it might be increasing the peripheral blood neutrophils, as Murugaiah , et al. findings that show that Properdin is released from the specific granules of activated peripheral blood neutrophils, which can directly interact with pathogens.

However, another study reported the opposite finding, as Alosaimi revealed that higher properdin levels are more likely in severe diseases and those with fatal outcomes (Alosaimi et al., 2021).

Indeed, levels of components (C1q, C4, C3, C5, C9) and regulators (FH, FI, properdin) were

decreased compared to moderate disease, suggesting that increased consumption outpaces production in severe disease. The observation that C1q and C4 levels were reduced in the severe disease implies increased classical pathway activation, perhaps driven by anti-SARS-CoV-2 antibodies (Jarlhelt et al., 2021; Kim et al., 2021).

This study has demonstrated that SARS-CoV-2 infection induced the deposition of complement molecules from the classical pathway (C1q, C3, C4), reflecting activation of the complement system of the classical pathway. Additionally, this study identifies that complement levels C1q, C3, and C4 can serve as predictive markers for illness development and unfavourable clinical outcomes among individuals affected by SARS-CoV-2 infection.

The study limitations can be mentioned briefly as the assessment of complement activation markers (C3a and C5a) in the serum of infected patients may reflect the hyperactivation of complement mediators C1q, C3, and C4 during SARS-CoV-2 infection.

Small number size for study groups and IgG and IgM level measurement.

5. **Conclusion:**

The C1q, C3, and C4 levels of the current study were considered lower in COVID-19 patients compared with healthy controls, implying that a lower level of classical pathway activation markers is a predictor of increased activation of the complement system of the classical pathway and consumption during COVID-19 infection, with the exception of properdin level, which found a non-significant difference between the study groups. The results may provide further support for other studies that identify the complement level changes in COVID-19-infected people.

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Ethical Approval

This study has received ethical approval no. 55297 from the ethical committee of the Iraqi Ministry of Health.

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