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# Study the Serum Complement C3, C4 and Some Hematology Parameters in COVID-19 Disease

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## Abstract

The COVID-19 pandemic has become a real challenge to the medical community. Along with the problem of treating patients in critical conditions caused by SARS-CoV-2, the pandemic COVID-19 has exacerbated some environmental, social and biomedical problems.This research included 90 patients with COVID-19 and 30 non-infected (positive control). All samples were collected from nasal swabs and pharyngeal swabs in subjects of different ages and gender from the period November 2021 to February 2022 and was performed at Baqubah General Hospital in Diyala. All samples (from infected patients and healthy patients) were already identified by using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). Complement C3, C4 and white blood cells, lymphocytes and platelets were determined for all samples. The current study indicates that the COVID-19 patients showed lower levels of C3 and C4 were (96.48 ± 6.72 and 22.03 ± 1.67) mg/dl respectively, while the hematology tests showed a lesser ratio of WBC and LYM were (11.94 ± 0.73 and 20.35 ± 1.46) µl, while a high ratio of PLT was (288.71 ± 12.71) µl.

**Keywords:** COVID-19, C3, C4, WBC, LYM, PLT

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# دراسة مصل المتمم C3 و C4 وبعض المتغيرات الدموية مرضى COVID-19

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## الخلاصة

أصبح جائحة فايروس كورونا تحديًا حقيقيًا للمجتمع الطبي. إلى جانب مشكلة علاج المرضى في الحالات الحرجة الناجمة عن السارس - CoV-2 أدى جائحة فايروس كورونا إلى تفاقم بعض المشكلات البيئية والاجتماعية والطبية الحيوية.

يشمل هذا البحث 90 مريضا مصابين بفيروس كورونا COVID-19 و 30 غير مصاب (السيطرة الإيجابية) وتم جمع جميع العينات من مسحات الأنف ومسحات البلعوم ومن أعمار وجنس مختلفة من الفترة من تشرين الثاني 2021 إلى شباط 2022 حيث أجريت في مستشفى بعقوبة العام في ديالى. تم تحديد جميع العينات (المرضى المصابين والمرضى الأصحاء) باستخدام تفاعل البوليميراز المتسلسل للنسخ العكسي. تم تحديد الخلايا التكميلية C3 و C4 وخلايا الدم البيضاء والخلايا الليمفاوية والصفائح الدموية لجميع العينات. أظهرت الدراسة أن مرضى COVID-19 أظهروا مستويات أقل من C3 و C4 كانت (96.48 ± 6.72 و 22.03 ± 1.67) ملجم / ديسيلتر بينما أظهر اختبار أمراض الدم نسبة أقل من خلايا الدم البيضاء والخلايا الليمفاوية كانت (11.94 ± 0.73 و 20.35 ± 1.46) ميكرولتر على التوالي بينما كانت النسبة العالية من الصفائح الدموية (288.71 ± 12.71) ميكرولتر.

**الكلمات المفتاحية:** فيروس كورونا C4، C3، خلايا الدم البيضاء، خلايا الدم الحمراء، الصفائح الدموية.

## Introduction

The modern coronavirus, (COVID-19), is an RNA virus that was discovered in Wuhan, China in December, 2019. It has a typical crown-like look under an electron microscope due to the presence of glycoprotein spikes on its envelope, and causes a 2% mortality risk [1]. This disease is caused by infection with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) zoonotic virus, which has recently emerged [2]. Patients with COVID-19 can develop pneumonia, severe ARDS symptoms, and multiple organ failure [3]. COVID-19 patients'

clinical features ranged from asymptomatic to severe respiratory distress syndrome and death in extreme cases. The most prevalent COVID-19 symptoms [4] are damage to the sense of smell, headache, cough, myalgia, nasal congestion, rhinorrhea, sore throat, nausea , asthenia, fever, diarrhoea and shortness of breath.

The complement system is one of the most significant components of humoral immunity, and it plays an important role in COVID-19 development [5]. Following innate immune activation via the alternative and lectin pathways, and acquired immunity activation via the classic pathway, the complement system exerts various protective effects against pathogenic pathogens. [6]. Immunoassays are commonly used in clinical repetition to determine and screen complement activation by measuring specific components, such as the complement proteins C3 and C4. Decreasing concentrations of serum C3 and/or C4 is a result of increasing product consumption [7,8]. Coronavirus infection is linked to coagulopathies and changes in the complete blood picture [9]. The examination of hematological and immunological parameter outlines in COVID 19 patients may aid in determining the clinical course of COVID 19 patients. Regular monitoring of hospitalized patients will help in planning treatment [10]. The aim of this study is to determine how well hematologic and immunologic indicators might distinguish COVID- 19 patients.

## Material and Methods

Ninety patients infected with COVID 19 (38 males and 52 females) were between the ages of 18 and 65 years. Thirty (11 males and 19 females) were non-infected with COVID 19 (positive control). The samples were already diagnosed by using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) from nasal swabs and pharyngeal swabs. This study was performed at Baqubah General Hospital in Diyala between November, 2021 and February, 2022. Each infected and healthy subject was tested for haematology (complete blood count) and immunological parameters (C3 and C4) as follows: complete blood count (C.B.C.): Using an auto analyzer manufactured by a Spanish haematology company (SPINCELL3) in blood cell count for all samples. Tests also included: white blood cells (WBCs), lymphocytes (LMY), and

platelets (PLT). Complement C3 and C4 serum levels were estimated by using a radial immunodiffusion plate assay according to the information provided by the manufacturer . Before handling the plate, the wells were filled with 5 µL of sample and/or controls and allowed to adsorb completely. The plate was put in a wet room for 72 hours. Reading: The precipitate ring was measured with a suitable ruler or measuring lens, but with a system that allows for a maximum error of 0.1mm. The assay range for C3: 26 -300 mg/dL and for C4: 7- 90 mg/dL.

## Results and Discussion

### 1- Distribution of Study Population According to Age

Patients from ages 18 to 65 were divided into four age categories. Twenty-eight, or almost half (46.6%) of the patients were in the 18-30 age group. A third of the subjects (20) were in the 31-41 age group. There were nine subjects (15%) in the 42-52 age group and three subjects (5%) in the 53-65 age group. In the control group, sixteen or just over half (53.3%) of the subjects fell into the 31-41 age group. There was just one case (3.3%) in the 53-65 group. The statistical analysis revealed highly significant differences among them as shown in table 1.

**Table 1:** Distribution of Study Population According to Age

| Age groups Years | Patients No (%) | Control No (%) | P-Value |
|---|---|---|---|
| 18-30 | 28(46.6 %) | 8(26.7%) | 0.0001 ** |
| 31-41 | 20(33.3 %) | 16(53.3%) | |
| 42-52 | 9(15%) | 5(16.7%) | |
| 53-65 | 3 (5%) | 1(3.3%) | |
| Total | 60(100%) | 30(100%) | |
| ** (P≤0.01). | | | |

**Significantly at 0.05, ** Significantly at 0.01 and 0.05.**

The results of this investigation revealed that the highest percentage (46.6%) of patients with positive COVID-19 is seen in the 18 – 30 category, and only 3 cases (5%) are seen in in people over 50, as shown in Table 1. The results were consistent with Liu *et. al* [11] in their assessment of 4880 confirmed cases of SARS-COV-2 infection in China using RT-PCR, which found the highest rates of infection in the young age groups: 482 cases in ages 18-29, 1097 cases in ages

30-39, 841 cases in ages 40-49, 1011 cases in ages 50-59, 886 cases in ages 60-69, and > 70 (563) years 563 cases in those over 70. Sarhan *et al.*, [12] discovered that the majority of cases in Iraq occur in people aged 29 to 50, with those aged 30-39 being the most infected. Younger adults, particularly those under 35, have a high incidence of SARS-CoV-2 infection in the community, according to Goldstein et al., [13], and the mortality rate in the very elderly is significantly higher than the elderly using serological tests. Xu *et al.*, [14] who found that the elderly are more susceptible to the disease's more severe manifestations, disagreed with our findings. According to Wu and McGoogan, [15] the casefatality rate for patients aged 70 to 79 years was 8.0 percent, compared to 14.8 percent for those aged 80 and up. According to Bialek *et al.*, [16] SARS-COV-2 is more common in the elderly and those with a high risk of ICU admission and mortality in the United States. According to another study [17], elderly patients (> 65 years) with comorbidities and ARDS have a higher chance of death. Age groups Years Patients No (%) Control No (%) P-Value 18-30 28(46.6 %) 8(26.7%) 0.0001 ** 31-41 20(33.3 %) 16(53.3%) 42-52 9(15%) 5(16.7%) 53-65 3 (5%) 1(3.3%) Total 60(100%) 30(100%) ** (P≤0.01). The elevated risk of COVID-19 infection among the elderly could be attributable to several comorbidities [18]. Apart from the negative clinical outcome, the elderly with heart disease had the worst results [19].

**2- Distribution of Study Population According to Gender**

The total number of the patients who were positive for SARS-COV-2 was 90, 38 (42%) of whom were males and 52 (58%), females. The control group was comprised of 30 healthy subjects, 11 (37%) males and 19 (63%) females, as presented in Table 2.

**Table 2:** Distribution of Study Population According to Gender

| GENDER | | |
|---|---|---|
| Study Group | Patients with COVID-19 N = 60 | Controls N = 30 |
| Total (%) | 90 (100%) | 30 (100%) |
| Male | 38 (42%) | 11 (37%) |
| Female | 52 (58%) | 19 (63%) |

Table 2 shows the total number of samples distributed between male and female subjects and indicates more females (58%) infected with COVID-19 than males (42%). The results were asymptotic to a study in China which showed the percentage of females and males were 51% and 49% respectively [20]. Also, current results are almost identical to a study done in 2021 [21] which indicated the rate of a male infection was 44% while the female rate was 56% and corresponded with the outcomes of Gholizadeh *et.al* [22] when he showed the percentage of male patients with COVID-19 was 41% and female patients was 59%.

These results disagree with Chen *et.al* [23] who found that males were more vulnerable to SARS-CoV-2 infection than females, with males at an 81 percent rate.

Adult of males over 55 years old, mainly those with underlying comorbidities, might be more likely to grow acute COVID-19. A study [24] found that among total confirmed cases, the infection rate in females over 55 was 51.1%. The result of the present study also agreed with the result done by [25] which found that, among 632 confirmed Korean SARS-COV-2 cases, the number of infected females (430) was more than half number of total confirmed cases. In the USA, a study [26] reported 463,259 (55.9%) were female.

**3- The Complement Levels in Covid-19 Patients and Healthy Individuals**

The results shown in Table 3 show the mean serum level of C3 in sera of a patient with SARS-COV-2 and control (96.48 ± 6.72 and 127.09 ± 3.99) mg/dl respectively. Statistical analysis shows there is a significant difference (P= 0.001). It is evident from the table below that there are significant differences (p= 0.001) between patients and control (22.03 ± 1.67 and 31.45 ± 1.45) mg/dl respectively in the concentration of C4.

**Table 3:** The levels of Complement (C3) and Complement (C4) among patients with Covid-19 and Healthy controls.

| GROUPS | N | COMPLEMENT (C3) (MG/DL) (MEAN ± SE) | COMPLEMENT (C4) (MG/DL) (MEAN ± SE) |
|---|---|---|---|
| Patients with COVID-19 | 60 | 96.48 ± 6.72 | 22.03 ± 1.67 |
| Controls | 30 | 127.09 ± 3.99 | 31.45 ± 1.45 |
| *P*-Value* | 90 | 0.001 | 0.001 |
| *P* value <0.05 | | | |

The current results shown in Table 3, indicate that the rate of C3 and C4 levels were lower in patients with CoV-19 than in the control group. Several studies agree with the present study. A study done by Zinellu and Mangoni, [27] showed that the serum concentrations of complement C3 and C4 were significantly lower in COVID-19 patients with more severe disease or who died during follow up when compared to those with milder disease or survivor status. The magnitude of the observed SMD values was -0.40 for C3 and -0.29 for C4. C3 levels were similarly shown to be lower in a study conducted by [28]. A slight level of C3 might be an alert to the admitted COVID-19 with extra management when compared to survivors and non-survivors of COVID-19 patients. For COVID-19 individuals, inhibiting the complement pathway could be a viable treatment option. In the deceased individuals, the decreases in C3 and C4 were more pronounced. After SARS-CoV-2 infection, reduced C3 and C4, as well as dysregulation of cell subsections and cytokines, can lead to death [29].

In an animal study, [30] discovered that COVID-19 infected C3-poor animals had inferior amounts of immune cells, monocytes, neutrophils, and cytokines than COVID-19 infected controls, and concluded that SARS-CoV was incapable of causing the same inflammatory response in the absence of complement as in wild-type mice. In COVID-19, lower C3 levels may contribute to thrombosis, inflammation, and organ damage [31]. Individual variability in C3 and C4 synthesis might occur in acute cases patients due to extreme activation of the lectins path or the classical path through specific anti-viral antibodies or immune developments [32]. An advance C4 and C3 consumption might occur in acute cases patients due to extreme

activation of either the lectins path or the classical path through specific anti-viral antibodies or immune developments.

**4- The Hematological parameters levels among Covid-19 patients and healthy Individuals**

The results shown in table 4 showed the mean serum levels of white blood cells (WBCs), lymphocytes (LYM), and platelet (PLT) of patients with SARAS-COV-2 and control were (11.94 ± 0.73, 20.35 ± 1.46, 288.71 ± 12.71 ) and (7.74 ± 0.51, 24.29 ± 0.97, 260.50 ± 12.85 ) respectively. Statistical analysis show there is a significant difference (P = 0.001, P = 0.028 , P = 0.165) respectively table 4.

**Table 4:** A levels of Hematological Parameters among Patients with Covid-19 and Healthy Individuals

| GROUPS | N | WBCS (ML) (MEAN ± SE) | LYM (ML) (MEAN ± SE) | PLT (ML) (MEAN ± SE) |
|---|---|---|---|---|
| Patients with COVID-19 | 60 | 11.94 ± 0.73 | 20.35 ± 1.46 | 288.71 ± 12.71 |
| Controls | 30 | 7.74 ± 0.51 | 24.29 ± 0.97 | 260.50 ± 12.85 |
| P-Value* | | 0.001 | 0.028 | 0.165 |
| *P* value <0.05 | | | | |

The current results in Table 4 shown the maximum rates of infected patients with increased levels of WBC, LYM and PLT with highly significant results. Several studies agree with current results [21] which found lower levels of LYM and higher levels of PLT in swab-positive cases, as well as a significantly decreased platelet count (P = 0.001). Previous investigations comparing RTPCR positive and RT-PCR negative patients found that the positive patient had a considerably lower lymphocyte count [33].

Abdulla *et al*., showed the highest rate of infected female patients, and 100% showed decreased lymphocyte count, while the male patients showed the highest ratio, 80%, of decreased lymphocyte count. Only 20% of infected male patients showed normal lymphocyte count while

the female patients revealed none [34]. The same study also found a highly significant relationship between COVID-19 infection and white blood cells count (WBCs) [34].A considerable rise in WBCs in patients with severe disease may signal clinical worsening and an increased likelihood of reduced outcome. It also implies that the increase in WBCs is driven by increasing neutrophils, as lymphocytes, monocytes, and eosinophils all showed declining trends. As signs for of probable progression to critical disease, doctors should closely monitor WBC count, LYM count, PLT count, serum ferritin and IL-6, [35].

Another study was carried out by Chen *et. al* [23] pointed that WBC counts were normal or slightly increased above the higher limit of normal (ULN) in all the acute cases. Another study [36], showed a highly increase of in WBC. The WBC rises in response to acute inflammation, which could indicate damage to the lungs and extrapulmonary organs, such as respiratory distress, acute heart injury, and acute kidney injury [37].

Young children, with mild COVID-19 had lesser lymphocytes in the acute phase, which was consistent with previous studies [38]. According to the epidemiology and clinical characteristics of COVID-19, young children under 6 years have the lowermost lowest morbidity rate, and very few young children with COVID-19 develop acute cases [39]. In young children, patients with mild COVID-19 had lower lymphocytes in the acute stage, which was consistent with previous studies [38]. Based on the epidemiology and clinical characteristics of COVID-19, children under six have the lowest morbidity rate, and very few young children with COVID-19 develop severe cases [39].

The following common haematological abnormalities were reported in the early reports: lymphopenia (82.1%), thrombocytopenia (36.2%), and leukopenia (33.7%) [40]. A low platelet count is frequently linked to a viral infection that isn't COVID-19 specific [8]. Similarly, the impact of low platelet counts in predicting prognosis has been extensively documented in a number of investigations involving critically ill patients, several studies have found that certain patterns, such as a low nadir platelet count or a rapid drop in platelet count, are significant [41]. Low platelet count has been confirmed as a significant predictive factor of severity and mortality in COVID-19 patients [42]. The presence of disturbed myelopoiesis in patients with

severe, systemic COVID-19 infection is suggested by alterations in circulating neutrophils and platelets [43]. Another theory [44] proposes that virus-induced autoantibodies destroy platelets, which subsequently form immune complexes and are excreted from the body. Platelet consumption during the coagulation cascade and thrombi formation, in addition to these possible activities, reduces platelet levels [45].

## Conclusions

COVID-19 has emerged as a healthcare issue unlike any other, posing a challenge to all medical disciplines due to widespread occurrence and unique clinical symptomatology and syndromes. It is essential that a hematology expert In address clotting disorders and associated cytopenias and lymphopenia. Lower C3 and C4 values, which indicate complement activation, were linked to higher COVID-19 severity and morbidity rates. C3 and C4 could be effective in predicting negative clinical outcomes in these patients.

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