Research article

Immunological inflammatory factors in patients diagnosed with COVID-19

Mohammed S. Abbas[1], Ahmed Ghaith Ahmed[2], Sura Qusay Ali[3], Bahaa Abdullah Lafttah AL-Rubaii[4]

[1]Pharmacy Department, Ashur College, Baghdad, Iraq
[2]Department of Prosthodontics, College of Dentistry, Al-Farahidi University, Baghdad, Iraq
[3]College of Education, Al-Iraqia University, Baghdad, Iraq
[4]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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Corresponding author: **Sura Qusay Ali.** Email: sorra.q.ali@aliraqia.edu.iq

ABSTRACT

Introduction and Aim: Due to the Coronavirus outbreaks, the SARS-CoV-2, also known as COVID-19, has claimed several lives around the world, with the majority of them being elderly, suffering from underlying chronic illnesses, or living in vulnerable conditions. This study aimed to find the immunological factors CD-79, CD-4, IL-2, and TNF-B in COVID-19 patients utilizing nucleocapsid-(N), a protein structure that interacts with genomic RNA to create complexes.

Materials and Methods: SARS-COV-2 infection was detected using RT-PCR. The serum levels of IL-2 and TNF-B, as well as the concentrations of CD4 and CD79, were measured. This study included 100 COVID-19 patients.

Results: The results showed that the serum concentration of TNF-β and IL-2 in COVID19 patients was significantly higher than that in the general population (with acute and moderate illness) when compared to normal control groups ($p<0.05$). COVID-19 patients reported higher levels of CD79 as well as CD4 expression than healthy control groups in a study of activated markers.

Conclusion: Infection with SARS-COV-2 has a high impact on various immunological and inflammatory markers in patients.

Keywords: Covid-19; nucleocapsids gene (N); RT-PCR; inflammatory factors.

INTRODUCTION

The coronaviruses are members of the virus family Corona-viridae (order Nido-virales) (CoVs). Coronaviruses can infect humans, birds, bats, snakes, mice, and many other species (1,2). Coronaviruses cause mild to severe illness and have been implicated in pandemics (3). CoV genomes are structured as follows: 5′-leader-UTR-replicase 3′-UTR poly (A) tail -S (Spike) -E (Envelope) -M (Membrane) –N (Nucleocapsid) UTR poly(A) -3′ tail Some of the accessory genes close to the 3' end of the genome (4) have been found to have significant roles in the development of viral pathogenesis (5). Coronavirus-induced SARS is caused by Covid-19 (SARS-CoV-2). The viral membrane shields SARS-viral CoV-2's DNA in nucleocapsids, a helical protein. Nucleocapsids are proteins. Polymerase chain reaction targets the nucleocapsid protein, which helps viral RNA transcription and replication (PCR). Critical CD8+ and CD4+ T-cell responses appear to be connected to recovery from severe illness. Antibodies-induced decline has been linked to chronic sickness, indicating that CD4+ play a key role in the management of this condition (6). When the virus penetrates a cell via endosomes and the Toll-like receptor-7, it can cause disease (TLR-7). Renin is the end product of the enzymatic process known as ACE-2, which converts angiotensin into renin. Cytotoxic CD8+ T cell production involves the presence of TNF-β, IL-12, and IL-6, all of which are produced in response to TLR-7 activation. Antigen-specific B-cell development and antibody synthesis by CD4+ helper T cells are both part of this process (7).

The majority of patients with COVID-19 had low white cell counts and lymphocytopenia; however, patients whose condition was more severe had considerably higher levels of neutrophils, dimer-D, and urea in their blood, in addition to a gradual drop in lymphocytes. These patients have been discovered to have high levels of a wide variety of cytokines and chemokines, including but not limited to IL-6, IL-10, and TNF-β, among others. Patients who were admitted to intensive care units had elevated levels of several cytokines and chemokines, including interleukin (IL)-2, IL-7, IL-10, macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (GM-CSF), intercellular adhesion molecule (ICAM)-1, and tumor necrosis factor alpha, compared to patients who were not admitted to intensive care units (8). Hence in this study, we aimed to look into the levels of CD-79, CD-4, IL-2, and TNF-β in COVID-19 patients and compare the levels in individuals negative for the infection.

MATERIALS AND METHODS

Patients

One hundred COVID-19 patients were signed up for the study. Patients with fever, cough, asthma, muscle aches, fatigue, and pneumonia like symptoms were subjected to Covid-19 detection by RT-PCR. The control group consisted of 70 COVID-19 individuals.

Sample collection

Blood samples were collected aseptically under standard laboratory conditions. The serum obtained was evaluated for cytokine levels (IL-2, TNF-B, CD-4, CD-79 and CD-54) by using ELISA (R&D Systems, country). The procedure was carried out according to the manufacturer. Using a microplate reader, the optical density (OD) was determined to be 450 nanometers (Beckman-Coulter).The serum cytokine concentration is expressed as picogram per milliliter (pg/ml).

Nucleic acid extraction

Using the nucleic acid extraction kit (Lifotronic, Shen Zhen, China), we isolated genomic RNA from 200 L of blood and processed it in line with the manufacturer's recommendations. A Nanodrop spectrophotometer was used to measure the concentration of the isolated RNA, and then the cDNA that resulted from the reverse transcription was purified. The DNA was then put through a Real-Time PCR experiment, where the COVID-19 virus was detected quantitatively utilizing probes specific to the virus. PCR recommended tools for detection (9,10). The reactions were performed in the following thermal-cycling conditions: denaturation at 95° C for 3 min, followed by 30 cycles at denaturation 95° C for 30s, annealing and extension at 60°C lasting 40 cycles.

Statistical analysis

The SPSS-19 program was used to conduct the statistical analyses. Chi-square (X^2) analysis was used to compare the data. P values less than 0.05 were judged to be statistically significant.

RESULTS

The Covid-19 patients in this study were grouped as having mild, intermediate or acute infection based on their clinical signs. Majority of the patients in the study had mild infection (75%), followed by intermediate and acute infection (Fig.1).

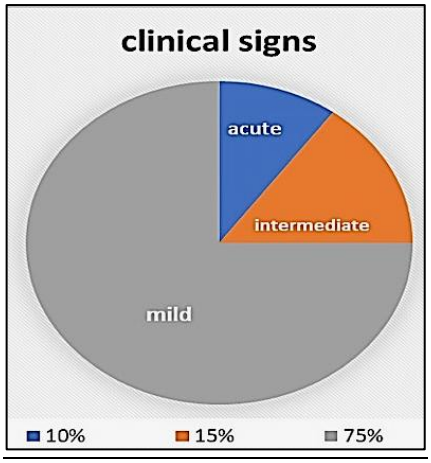

Fig. 1: The distribution of COVID-19 infected based on the severity of infection.

RT-PCR detection of COVID-19 infection

RT-PCR using *nucleocapsids (N*) gene specific primers was also used to establish the presence of covid-19 at the molecular level. Fig. 2 shows a Log graph representing an amplification of the *nucleocapsids* (*N*) gene. A positive RT-PCR for Covid-19 was found in every patient.

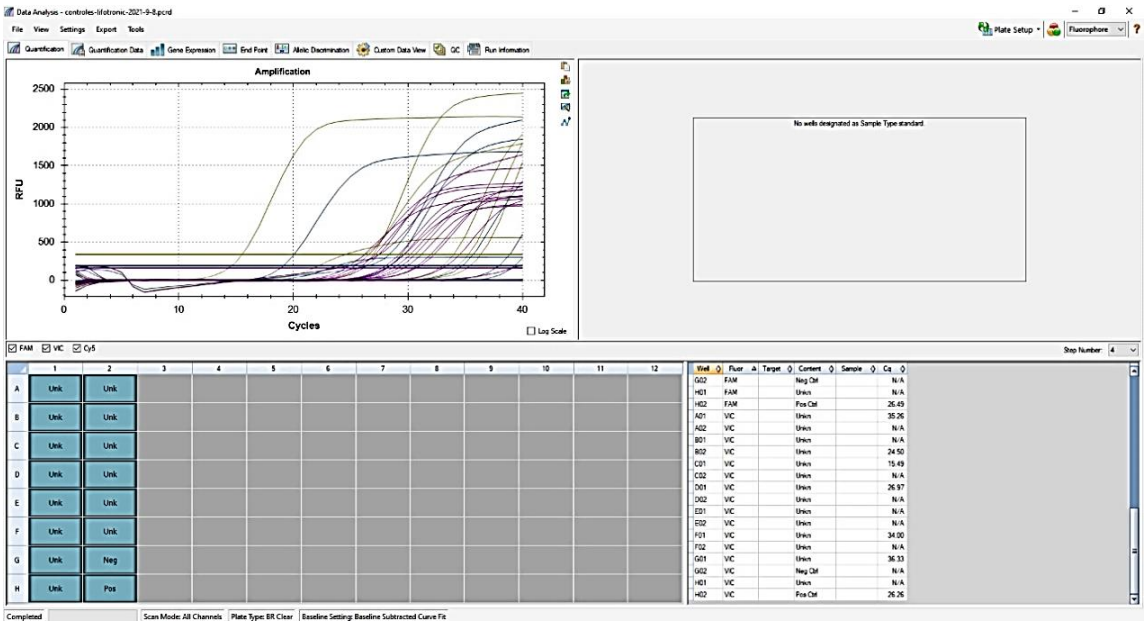

Fig. 2: RT-PCR technique amplification of nucleocapsids (N) gene with Log graph type

Serum concentration of IL-2

Individuals diagnosed with COVID-19, as well as those with severe or moderate disease, frequently reported higher levels of IL-2 in their blood than healthy control subjects did. In comparison to the patients in the control group, those who were diagnosed with a moderate to severe illness had significantly greater levels of the cytokine IL-2. A comparative analysis, however, revealed significant differences between the COVID-19 and healthy control groups ($p<0.05$). Similarly, serum IL-2 levels also showed significant differences ($p<0.05$) between the groups with mild, intermediate, and acute illness. The t-test revealed significant arithmetic differences between the transitional, severe illness, and mild disease groups ($p<0.05$; Fig. 3).

Serum concentration of TNF-β

TNF-B levels have been reported to be greater throughout in all patients with COVID-19 than in normal controls, and TNF-β concentration improved in acute COVID-19 cases, mild patients, and moderate patients, respectively. Between acute, moderate, and control persons, analysis of variance ($p<0.001$) was performed. There was a statistical difference significantly between the acute COVID-19 moderate as well as mild illness groups ($p<0.001$), according to the test (Fig. 4).

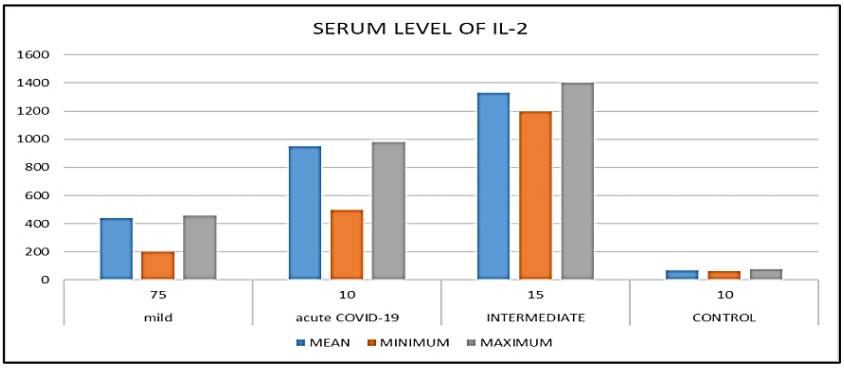

Fig. 3: Serum level of IL-2 in mild, intermediate, acute SARS-Cov-2 patients, and the control group.

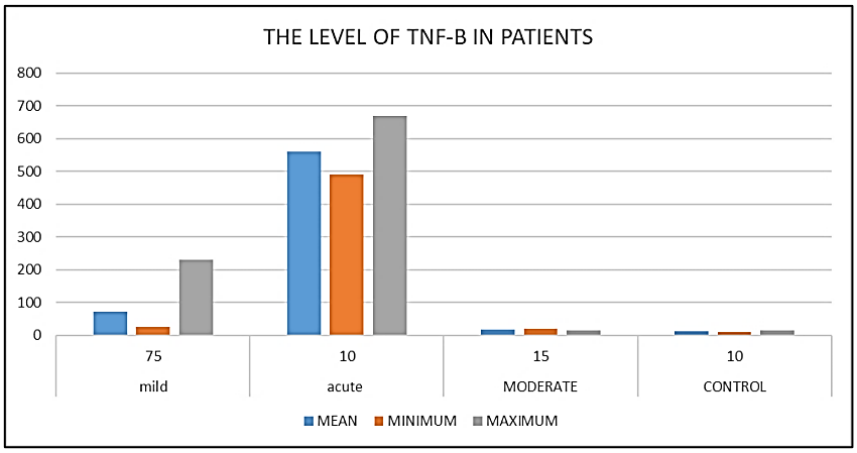

Fig. 4: TNF-B levels in patients and controls

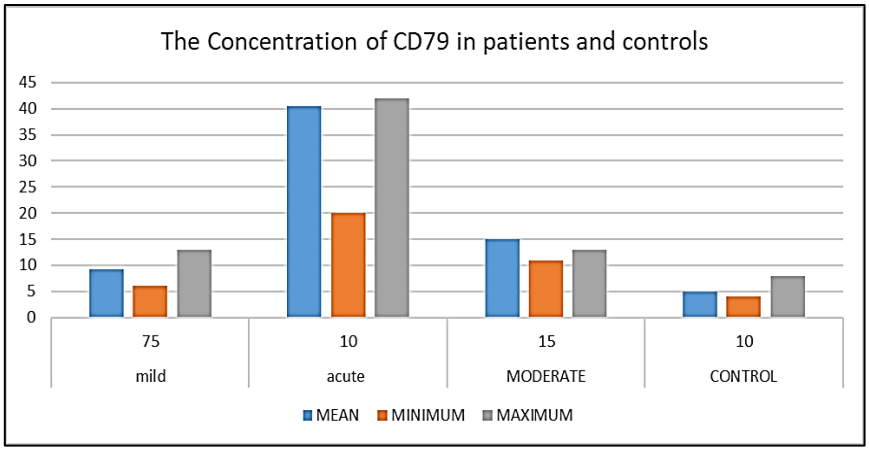

Fig. 5: CD79 levels in Covid-19 infected and healthy groups

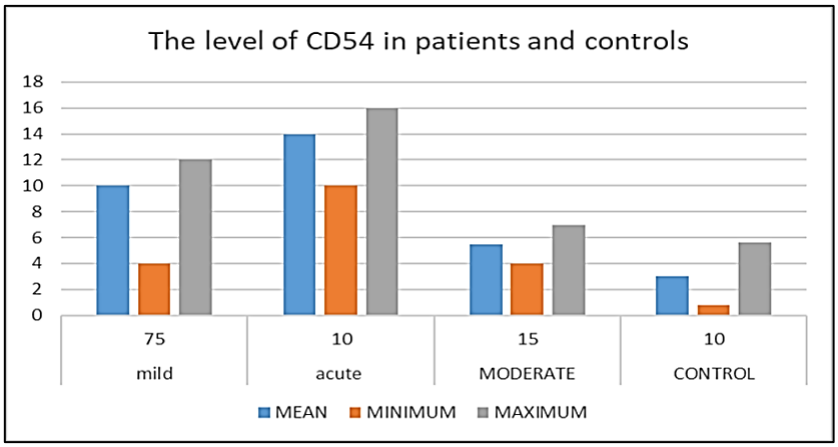

Fig. 6: CD54 levels in Covid-19 patients and healthy individuals

Serum concentration of CD-4

Results showed that the mean CD-4 concentration in patients and normal healthy-control groups differed significantly ($p<0.05$), and that the cell membrane of CD-4 was overexpressed in the order of acute, mild, and normal control groups.

Serum concentration of CD-79 and CD-54

Our results shows that the mean CD-79 and CD-54 concentration varied considerably ($p <0.005$) between COVID-19 patients and normal healthy control groups, and that CD-79 and CD-54 cells were overexpressed in acute COVID-19 cases, mild instances, and normal control groups, respectively. (Fig.5 and Fig.6)

DISCUSSION

In terms of the clinical symptoms, our research found that ten percent of the cases had fever, dyspnea, myalgia, and tiredness. Fifteen percent of the cases had moderate signs of infection, and seventy-five percent of the cases had a mild sickness. The results of the T-test indicated that there was a statistically significant change in the clinical indications ($p<0.05$). Patients with Covid suffered a handful of symptoms that were prevalent, such as fever and weakness.

Patients with COVID-19 can suffer devastating consequences due to the immune dysfunction associated with elevated cytokine storm (11). In most fatal cases, it was found that excessive activation of cytokine storm was responsible due to which there was development of acute lung injury and then acute respiratory distress syndrome (ARDS) (12). Even in severe and critically ill cases of COVID-19 patients, the pathological changes were related to activation of cytokine storm (13). Throughout previous two years, numerous researches had addressed the pathogenesis route of COVID-19 infections and reported many markers associated to cytokine storms. Most study groups reported G-CSF, GM-CSF, IL-1, IL-5, IL-6, IL-7, IL-8, IL-10, IL-15, IL-27, TNF-α, MCP-1, C-reactive protein, ferritin, LIF, MIF, and VEGF (14). In our present study, we studied the level of the interleukin-2 (IL-2) and tissue necrosis factor-beta (TNF-β) in different stages of COVID-19 cases. IL-2 was significantly increased in both acute and moderate COVID-19 patient. However, TGF-β was significantly increased only in acute COVID-19 patient. The level of TGF-β in mild and moderate COVID-19 was insignificant in comparison to healthy volunteers. In early 2020, Huang et al., 2020 reported the immunological profile of COVID-19 patients in Wuhan, China (15). They found that COVID-19 patients in the ICU had higher levels of IL-2 than those outside of the ICU. This increase in IL-2 in intensive care unit patients exemplifies IL-2's function as an anti-inflammatory cytokine that can halt the worsening of an existing condition. With its effects on IL-6-dependent signaling events and its inhibition of TH17 differentiation, IL-2 is widely recognized as a potent anti-inflammatory agent (15). In presence of IL-2, TGF-β help in the differentiation of induced Treg cells (14). In the presence of IL-6 and IL-4, TGF-β increases the differentiation of T helper cells and Treg cells in a concentration-dependent manner. This in turn increases tissue inflammation and promotes autoimmune condition (14).

Chemokines are proteins that play a significant part in the treatment of viral infections. They do this by bringing in both innate and adaptive immune cells to the site of the infection, and by boosting the immune cells' ability to release antiviral mediators and cytotoxic effects (11). The chemokines they produce allow some viruses to evade the body's immune system and thrive (16). Chemokines have been discovered to activate NK cells, which then destroy infected cells in situations of viral infections like vaccinia and cytomegalovirus (13). Another benefit of chemokines is that they stimulate natural killer (NK) cell lysis of tumor cells (14). The white blood

cells known as neutrophils serve as the body's initial line of defense against infection. The cytokines including IL-8, IL-10, and IL-12 secreted by these cells, lead to the production of reactive oxygen species. Increased neutrophil activity in the lungs is triggered by IL-8 release (15). The inception of IL-8 can also be increased by linking the (TLR7 and TL-R2/ TL-R3) receptors which differentiate portions of the infection multi-protein, double-strand-RNA, as well as against viral assemblage of the host individually (16). In cases of acute infection, both the innate and adaptive immune systems respond effectively to COVID-19. Many processes, including tumor morphogenesis, irritation, angiogenesis, apoptosis, invasion, proliferation, and metastasis, have been linked to the development of an effective adaptive T-cell response that generates both cytolytic TNF. We found that acute COVID-19 patients also had substantial increases in CD54 and CD79. Wang et al., 2020 had shown the decreasing pattern of monocyte counts in deceased patients as compared to surviving COVID-19 patients (17). In deceased COVID-19 patients, CD54 positive cells reduced from early onset to late stage. In COVID-19 survivors, CD54 levels rise from early to late infection. In mild and acute stages, CD54 increased, but in moderate stages, it was similar to healthy volunteers. Escher et al., 2020 found that COVID-19 patients with myocarditis have higher CD54/ICAM-1 levels (18).

TNF alpha binds to receptors on cell membranes, where it exerts its effects. TNFR-1 (55 kDa) and TNFR-2 (75 kDa). These two receptors are a part of the group of receptors known as TNF receptors. The direct induction of other supporting incendiary cytokines is responsible for TNF- α favorable to fiery effect. The cytokine storm of a severe SARS-CoV-2 infection will set off the powerful round via the body's protective framework, leading to (acute respiratory distress syndrome) and organ failure, and ultimately death (19).

According to Fig. 5, there was a statistically significant difference (p 0.05) between the mean CD79 expression of COVID-19 patients and that of normal control groups. The control groups, mild patients, moderate disease, and acute patients all had the greatest rates of CD79 expression. COVID-19 elimination is linked to a flurry of multivalued CD4+ and CD8+ T-cells responses, whereas populations with persistent infections are more likely to have frail, thinly committed responses (20). CD8+ TCM cells appear to be on the verge of converting to EMC cells, which have been enlisted to the body in COVID-19 afflicted persons (19) was used to prove that CD8+ effector cells in the body have decreased usable ability. Antigens from the fragile 'CD8+ T cell response' are widely used to determine liver pathogens (20, 21). In our attempt to determine the pathogenic status of CD79 due to the viral infection showed a strong upregulation among both CD4 as well as CD79 which mark lymphocytes within peripheral blood of COVID 19 people in the form of immune dysregulation (22).

CONCLUSION

Our study concluded that different immunological and inflammatory markers in patients are significantly impacted by SARS-COV-2 infection.

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

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