



Assessment of alterations in IFN-g and IL-17 levels in COVID-19 patients

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Abstract

The current thesis aims to examine changes in IFN gama and IL-17 levels in coronavirus patients in order to identify the most effective techniques for following up with patients and, as a result, offering the best medicines in the shortest amount of time. NRL, LYM, and WBC exhibited evident substantial alterations that might be utilized to detect the disease early or to monitor the patient's condition by comparing them to other blood parameters with unique differences. IFN-g, assessed by an operational immunoassay, was studied as an indicator for the likelihood of hospital throughout the initial phases of illness as well as one month following infection. Low concentrations of IFN-g and IL-17 were associated with higher hospitalization rates ($p < 0.001$). The study's main goal was to learn more about the function of mediation in COVID-19 patients. Cytokines like IFN- γ and IL-17 were found to be elevated in patients infected with SARS-CoV-2. These cytokines are crucial markers of sickness severity and could be utilized as therapeutic targets.

Key words: Corona virus 2019, SARS, IFN gama, IL-17 levels, NRL, LYM, WBC

Introduction

The SARS-CoV-2 virus was discovered in Wuhan, China, in December 2019. This virus is the third to kill individuals in the last 20 years, after SARS-CoV-2 in 2019, Middle East respiratory syndrome coronavirus (MERS) in 2012, and SARS-CoV-1 in 2002 (2). Since the outbreak started on May 25, the virus has infected 5,370,375 people and killed 344,454 of them. It has already spread to 216 countries, provinces, and territories. On March 11, 2020, the World Health Organization proclaimed SARS-CoV-2 to be a pandemic (3). Identification of WBC: A blood test known as a complete blood count (CBC) determines a patient's white blood cell count. An infection or inflammation may be indicated by a higher-than-normal white blood cell count. The presence of a low white blood cell count suggests that the person's immune system is malfunctioning. Patients with cancer and those on immunosuppressive drugs frequently experience this issue. The lymphocytes: The body's defenses against disease are strengthened by lymphocytes, which move throughout the body and produce proteins and antibodies that target infections and illness causes. While B cells fight off invasive germs and viruses, their T lymphocytes target cancerous or virus-damaged tissues (4). Naturally, the lymphocyte count was higher in asymptomatic Covid-19 patients. By generating antibodies and eliminating damaged cells, this clearly shows how well their immune systems are combating the coronavirus (5). Neutrophils are immunological cells that produce between 50 and 70 percent of white blood cells. Neutrophils are the first cells to respond when the body becomes polluted, and they also play a role in chronic infections. It prevents fungal and bacterial infections, but not viral ones. (6) Despite the lack of



evidence to support their function, it has been suggested that those cells improve antiviral defenses by interacting with other immune system cells, which results in the manufacture and breakdown of cytokines, eating the infecting virus, and initiating an oxidative explosion. Pre-existing immune inadequacies can be accommodated by 7IFN activity in the early stages of SARS-CoV-2 infection, even before damage begins, in people who may develop a severe form of the sickness. Controlling the baseline IFN-g response, as determined by a straightforward functional immunoassay, is therefore anticipated to result in a greater number of hospitalized severe COVID-19 patients. A useful blood test for identifying people with an unbalanced IFN-g reaction can elevate authority by directing the treatment of antiviral drugs, IFN, and/or an antibody with therapies for those who are likely to benefit from them and lowering hospital stays while refusing harmful overprescription and potential issues in those who are unlikely to benefit (8). Interleukins (ILs), chemokines, and interferons are some of the cytokines associated with COVID-19. The development of epithelial cell immunity and the host's defense against bacterial and fungal infections are associated with T-helper 17 (Th17) cells' generation of IL-17. IL-17 increases antibacterial chemicals, chemokines that speed up processes, and inflammatory responses in its two different forms, IL-17A and IL-17F. Recent research indicates that COVID-19 patients generate more IL-17, which heightens inflammatory reactions. This study evaluates the properties of IL-17A, how it relates to COVID-19, and whether an IL-17A-based treatment is feasible (9).

Materials and Methods

Design of study

After approval, 150 individuals spanning in ages between 25 to 101 was chosen. These instances range from mild to severe injuries, and there are (50) samples from persons who lack the condition to compare, As mentioned in the part below. The research was conducted at Jalawla General Hospital, particular in the medical hall for the tests that were available to the a hospital, and the testing occurred in its labs in the medical Chemistry division, with the balance of the complicated tests performed in a specialized laboratory after samples were collected from clients, divided, frozen, refrigerated, and sent to the laboratory in Diyala, Iraq. The trial spanned a period of three months between the first of March to June 1, 2022.

Sample collection

Based to the pulmonologist's reports and the kinds of specimens driven, each patient had 10 ml of venous blood taken using a variety of tubes. Blood samples were allowed to clot on a plate at room temperature for fifteen minutes before being spun for ten minutes at 3500 rpm. The blood was then



separated from the left over cells. The blood sample was taken out with a little syringe and kept in an ordinary tube in the freezer.

Methods

Complete blood count (CBC)

A comprehensive blood count was carried out to determine the amount of hemoglobin (Hb), white blood cells (WBC), and blood platelets. The ERBA560 analyzed blood samples in minutes. Put a blood specimen in an EDTA vial. Place the vial in a spinning shaker for 3 to 5 minutes. When the rotation's moments is up, gather the blood examples of and in it in a complete blood count equipment (ERBA 560). After a few minutes, the machine examines a blood collection and shows the WBC count.

Immunoassays

Nurses and physicians took blood samples in vials containing lithium heparinate at 8 a.m. and 12 p.m. In QuantiFERON-Monitor® specialized tubes (Qiagen®, Germany), one milliliter of whole blood was treated with immune agents that mimic molecular patterns linked to pathogens that activate immune cells within eight hours of collection. Using the QuantiFERON® SARS-CoV-2 test (Qiagen®, Germany), we collected a milliliter of blood from each individual in tubes containing a mixture of SARS-CoV-2 peptides in order to measure the quantities of IFN-g and IL-17 generated by SARS-CoV-2-specific T cells. Both SARS-CoV-2-specific and nonspecific immunological agents have been used to prime the specimens. Samples were spun at 2000-3000 x g for 15 minutes after being incubated at 37°C for 16 to 18 hours in order to collect plasma. In order to maintain sample quality, plasmas were then kept at -80°C until analysis, undergoing as few freeze-thaw cycles as possible. After excitation, an enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of plasma IFN-g and IL-17. A nonspecific response is produced by the approach. It should be mentioned that IFN-g levels found following a general reaction were higher than the detection limit (i.e., IFN-g >1000 IU/mL). A random score of 1000 IU/mL was applied to the data. This function testing is appropriate for routine use since it is easy to administer to both laboratory staff and doctors. Additionally, we used ELISA with physiological entertainment and specially made Ella cartridges to quantify plasma IL-17 for participants who were randomized through doctor consultations (CovImmune 1 study).

Results

The Results of Serum IFN-g, IL-17 and hematological Parameters in COVID-19 patients



Table 1 shows that Covid-19 illness has a clinically significant and statistically significant impact on the averages of IFN-g, IL-17, NRL, LYM, and WBC.

Table 1 The average serum IFN-g, IL-17, and hematological parameters in COVID-19 patients

Test	Groups	N	Mean	Std. Deviation	Std. Error Mean	P
IFN-g	Group A	50	15.215	6.05180	.85563	.000
	Group B	150	6.930	2.3221	.49814	.000
IL-17	Group A	50	2.871	5.41868	.76666	.000
	Group B	150	32.975	5.57881	.45812	.000
NRL	Group A	50	7.0260	1.21468	.17292	.000
	Group B	150	83.424	1.78565	.14811	.000
LYM	Group A	50	5.4980	2.50290	.33992	.000
	Group B	150	7.7579	1.39817	.11346	.000
WBC	Group A	50	3.8351	.62172	.08839	.000
	Group B	150	7.8425	5.56118	.37357	.000

Results of D-Dimer and Ferritin among individuals with COVID-19.

Our study's findings showed that elevated D-Dimer and ferritin levels among COVID-19 patients had statistical significance ($P < 0.05$), high moral differences, and substantial clinical benefit. According to the results, these markers are useful and can be used to track a person's health throughout the course of an infection.



Figure 1 shows the mean Ferritin levels in COVID-19 patients.

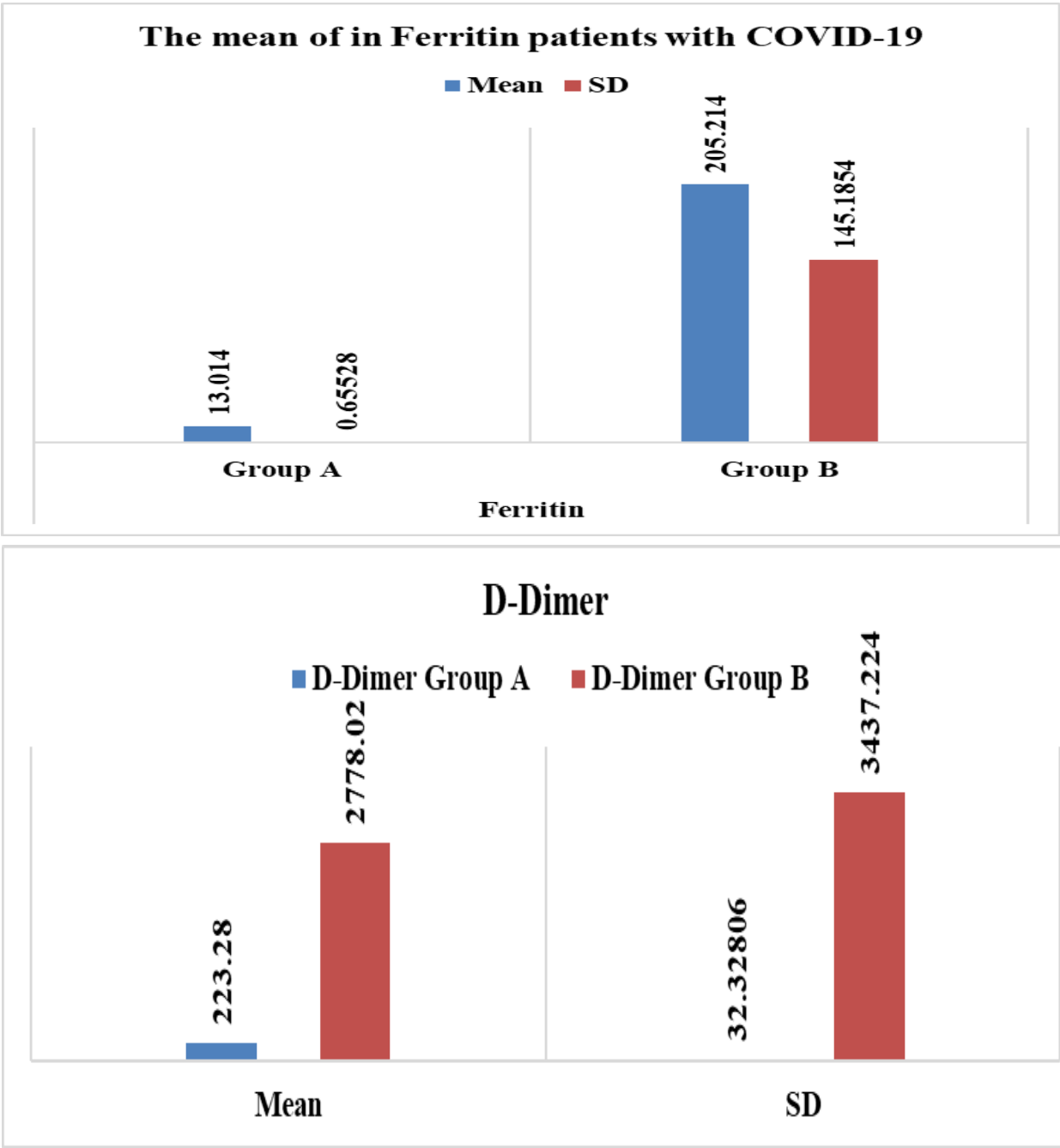


Figure 2: The average of D-Dimer levels in COVID-19 patients

Discussion

When compared to other blood tests, NRL, LYM, and WBC showed distinct, noteworthy variations that could be helpful in monitoring a patient's health or identifying illnesses early. Our study, which outlines the D-Dimer and Ferritin pathways, provides fresh understanding into the process that causes the shift in these values in Covid-19 patients, which aids in the early diagnosis of the disease because the results of these tests



vary widely. It was notably higher than that of the control group. D-Dimer and ferritin are significant markers of COVID-19 disease. (10) Daily complete blood counts (CBCs) with quantitative WBC separation should be performed on hospitalized COVID-19 patients in order to look for morphologic and numerical alterations that might indicate a bad prognosis and the advancement of the illness (11). The WBC count upon admission is highly correlated with death in COVID-19 patients. Prioritize high WBC levels when treating COVID-19 (12). We examined the cytokine levels in COVID-19 patients' serum at different stages of the illness's progression. COVID-19 markedly increased IFN- γ and IL-17 levels. These findings are consistent with past research suggesting that more severe or even fatal COVID-19 episodes may result in elevated Th1 and Th17 systemic immune responses (13). The findings suggest that CD4⁺ T cells may overproduce responder cytokines Th1 and Th17, which may be the underlying factor contributing to COVID-19's severity. This is consistent with previous findings that CD4 cells and other immune cells may change after severe COVID-19 infections [35]. This work, however, is the first to identify the exact mechanism that causes hyperinflammation, which in turn raises the risk of COVID-19 (14). Increased transcriptional activity of CD4⁺ cells triggers pathogenic Th1 and Th17 immune responses, which result in an excess of inflammatory IL-12, IFN- γ , and IL-17. By disrupting the immune response's equilibrium, this could cause the illness to go into remission. Instead, high levels of proinflammatory Th1 and Th17 cytokines increase circulating cytokine levels, disrupting the immune system's balance and decreasing its efficacy, allowing COVID-19 to spread. More research is needed to identify the likely molecular pathways that lead to the rapid, uncontrolled, and hazardous overactivation of the cytokine immune response in order to propose new therapy options for SARS reduction. CoV-2 infection (15).

The development of SARS-CoV-2 depends on the human immune system. The different cytokines influence the infection's progression and severity. These findings may further our knowledge of the pathophysiological function of hormones as well as their potential applications in illness prognosis and treatment. As previously reported, our data demonstrates that IFN- γ is a strong predictor of illness severity. Furthermore, it was shown that IL-7 levels were inversely connected with the severity of the condition and were lower in sick people than in healthy people. Finally, in order to better understand the role of cytokines in SARS-CoV-2 pathogenesis, the study intends to characterize the levels of various cytokines in patients with critical to severe sickness (16).

Conclusions

The study's main objective was to comprehend the function of mediation in COVID-19 patients. Cytokine levels, such as those of IFN- γ and IL-17, were elevated in patients infected with SARS-CoV-2. These cytokines may be used as therapeutic targets and are important markers of the severity of sickness.



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