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Immune Insights: Profiling the Response in COVID-19 Affected Individuals

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Abstract:

Background: The rapid spread of the COVID-19 pandemic, combined with the varying severity of the disease obviously, has prompted researchers to begin searching for possible indicators of disease outcomes. Therefore, the aim of the present work was to assess the immune response in COVID-19 patients in comparison with controls groups.

Methods: We investigated several immunological measures, including markers of innate immunity as Interferons (IFN) and adaptive immunity as immunoglobulin and lymphocytes, in 50 individuals with COVID-19 illness in comparison to 50 health control. Cytokines were measured in each sample using a custom Milliplex panel. Serum IgG and IgM were detected using the Milliplex MAP SARS-CoV-2 Antigen Panel

Results: lymphocyte counts were significantly lower in patients ($0.78 \pm 0.29 \times 10^3/\mu L$) compared to controls ($2.85 \pm 0.98 \times 10^3/\mu L$; $p = 0.001$). Interferon gamma-induced protein 10 (IP-10) levels were markedly elevated in patients, with a mean of 493.3 ± 142.32 pg/mL versus 259.9 pg/mL in controls ($p = 0.005$). monocyte chemoattractant protein-1 (MCP-1) levels were significantly higher in the patient group, (578.94 ± 145.32 pg/Ml) compared to (365.54 ± 120.01 pg/mL) in controls ($p = 0.015$). Tumor necrosis factor-alpha (TNF-α) also demonstrated significant elevation in patients, with a mean of 143.7 ± 15.65 pg/mL compared to 37.0 ± 14.52 pg/mL in controls ($p = 0.047$). There was a decreased concentrations of serum PD-1 on CD3+CD4+ and CD3+CD8+ in the Control group compared to COVID-19 with statistically significant difference ($p = 0.001$* and 0.041*respectively).

Conclusion: The immune system plays an important role in slowing the progression of COVID-19 to more serious stages of the illness. SARS-CoV-2 infection activates both innate and adaptive immune responses. The development of immune-modulating medicines that seek to stop or regulate the cytokine appears to be the most effective available weapons against COVID-19.

Keywords: immune response, lymphocytes, adaptive immunity, COVID-19

Introduction

Coronaviruses (COVID-19), a global pandemic, are single-stranded RNA viruses that caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is mostly transmitted in humans by respiratory droplets, as replicating SARS-CoV-2 has been detected in both the upper and lower respiratory tracts [1&2]. SARS-CoV-2 penetrates the host and infects target cells, replicating and spreading new virus particles [3].

The Middle East Respiratory Syndrome coronavirus (MERS-CoV) and the severe acute respiratory syndrome coronavirus (SARS-CoV) are both causal agents of COVID-19 [4]. The infection is transmitted from person to person [5] and through contact with infected droplets produced into the environment by coughing and sneezing, as well as contact distributed through the mouth, nasal, and ocular mucous [6]. Common symptoms of COVID-19 infection include fever, dry cough, weariness, difficulty breathing, bodily pain, chills, a sore throat, headache, weight loss, gastrointestinal disturbance, diminished smell and taste, allergic reactions to the skin, and discoloration of toes and fingers [7–8].

From March 15th to March 30th, 2020, there was a 4.7-fold increase in reported global cases, bringing the total global cases from 167,500 to 782,400 in a matter of weeks. During this time, the United States experienced extraordinary viral spread, with over 25,000 new cases reported per day in early April [9-10]. Three months later, on July 31, 2020, the United States (4,495,612 cases), Brazil (2,610,102 cases), and India (1,638,827) had the highest reported total number of COVID-19 cases, with the United States having nearly twice as many as any other country. The global mortality toll for COVID is 673,936, with the highest

death rates reported in the United States, Brazil, and the United Kingdom [11-12].

Statistics from the global outbreak show that COVID does not affect all patient populations similarly, with some experiencing asymptomatic infection and others developing serious disease with breathing difficulties and multiple organ dysfunction [7–8]. The pandemic was propelled by greater susceptibility among aging populations, among other causes, as the virus spread to Europe and the Americas [11]. By the first week of March 2020, the number of reported cases in China had dropped significantly to less than 500 per day, while other nations, including Italy, Iran, the United States, and Spain, had surged exponentially to 40,000 new cases per day by the third week of March 2020 [11-12].

SARS-CoV-2 has developed several variations, including Alpha, Beta, Gamma, Delta, and Omicron, which have spread over the world throughout the last two years. Mutations found in several SARS-CoV-2 variants determine the variant's tissue tropism. For example, the Omicron variation has greater receptor specificity for human ACE2. The Omicron mutations responsible for this high binding capacity with human ACE2 include Q493R, N501Y, S371L, S373P, S375F, Q498R, and T478K [13]. This distinguishing trait of Omicron could suggest that more attention needs to be placed on the early stage of sickness, when the virus replicates persistently.

When analyzing the Delta variation, the therapeutic impact of distinct strain tropism must also be considered. The Delta variant, with a tropism suited for the lower respiratory system has been identified as a lethal variant comparing to variants with tropism for the upper respiratory tract causing inflammation in the lower respiratory tract that immediately impairs lung function and is potentially fatal [14].

Overall, there is minimal evidence that the virus is primarily responsible for illness heterogeneity, leaving the host and environment as variables determining disease progression and outcome. The host appears to be the most important factor explaining disease severity, infection rates [15], and long-term medical consequences [16].

Because of a lack of immunity to SARS-CoV-2, disease severity and fatality rates are much higher in the elderly. It is probable that cross-

protection through adaptive immune responses against endemic coronaviruses contributes to moderate illness courses in young people. An effective immune response against SARS-CoV-2 involves the two parts of the immune system: the innate immune system, which includes granulocytes, monocytes, and macrophages, among other cells, and the adaptive immune system, which includes T and B cells [14].

Many published observational clinical investigations have found indications of immunological abnormalities linked to the extent of the disease and death in COVID-19 patients. Nevertheless, these studies showed significant variability in demographic variables, genetic traits, and therapeutic regimens prior to hospital admission [17].

The immune response to COVID-19 infections occurs in two phases [1]. The first phase is defined as immune protection-based and immunity aggravation [18]. A healthy person with human leukocyte antigen (HLA) should elicit an antiviral immune response during its period of incubation and in the non-severe stage [19]. The second phase consists of widespread inflammation and immune suppression [5]. A cytokine storm (CS) is a severe form of macrophage activation syndrome (MSA) or secondary hemophagocytic lymphohistiocytosis (sHLH) that causes tissue and organ dysfunction [1, 17].

Innate immunity is the primary line of protection against germs. It is made up of various biological components, including macrophages, natural killer cells, monocytes, dendritic cells, and neutrophils. The complement system, together with the coagulation-fibrinolysis system and interferons, all contribute to the innate immune response [18-20].

Therefore, in early June, the FDA recommended makers of licensed and authorized COVID-19 vaccines to use monovalent JN.1 vaccines (2024-2025 formula). Based on the continued evolution of SARS-CoV-2 and the rise in COVID-19 cases, the agency concluded and informed manufacturers that the recommended JN.1-lineage for the COVID-19 vaccines (2024-2025 formula) is the KP.2 strain, if possible [21].

The updated mRNA COVID-19 vaccines include Comirnaty and Spikevax, which are both approved for people over the age of 12, as well as the Moderna COVID-19 Vaccine and Pfizer-BioNTech COVID-19 Vaccine, which are both approved for emergency use in people aged 6

months to 11 years [22].

Literature Reviews

Interferons (IFN) Is the primary first-line defense against viruses. IFN types I and III, in particular, are key defense mechanisms. It has been shown that SARS-CoV-2 infection can cause dys regulation of the immune response mediated by IFN. Some investigations have indicated that patients with severe COVID-19 disease have a reduced or absent IFN type I activity (defined by the absence of IFN-β and low activity of IFN-α), in comparison with those with milder and moderate forms of the disease [21-23].

Gralinski et al., have recently been shown that SARS-CoV-2 nucleoprotein dimers was to activate mannose-binding protein-associated serine protease 2 (MASP-2), the primary trigger for activation of the complement system's lectin pathway. This results in C3 convertase and the membrane attack complex [19]. However, inhibiting either the nucleoprotein MASP-2 interaction or complement activation resulted in less lung injury [24].

Triggle et al, 2021 reported that Chemokines produced during COVID-19-ARDS attract neutrophils to the site of infection, as evidenced by transcriptional analysis of bronchoalveolar lavage fluid from patients with elevated CXCL-2 and CXCL-8. Neutrophils at the infection site release proinflammatory mediators such as cytokines (e.g., interferon-α, interferon-β, tumor necrosis factor, and interleukins 1β, 6, and 10) and chemokines (e.g., CXCL10), which contribute to COVID-19 development [25].

Antibody responses to COVID-19 are critical for virus clearance. Antibody maturation improves the body's defense against SARS-CoV-2 infections [26 -28]. Serum was analyzed many months after infection and shown to have modest antibody levels specific for single variants of SARS-CoV-2, but significant levels of antibodies capable of detecting the common epitope of numerous variants [29].

Kurahashi et al., performed further investigation using plasma collected 1-10 months after SARS-CoV-2 infection found that antibodies initially only protected well against the original variant that the patient was infected with, but plasma collected further away from the initial infection showed greater protection against variants of concern (VOCs). This suggests that,

while the total amount of antibody in serum may be decreasing, the protection provided against various forms of SARS-CoV-2 infection may not be decreasing significantly, if at all [30].

Cox et al., discovered that a tiny number of mutations could allow an escape from immune neutralization. It is important to note that this study was conducted only a few weeks after vaccination, so the antibodies did not have as much time to mature. Additionally, not all individuals received the full schedule of vaccination doses that is advised, and only half of the VOCs examined were even able to partially escape neutralization from the vaccine induced humoral immunity [31].

Painter et al., demonstrated that, whereas antibodies created in response to moderate COVID-19 infection have a 21-day half-life, memory T and B cells established retain their protective properties against identical strains of SARS-CoV-2 for considerably longer. Regardless of infection status, CD4+ T-cells increased soon following vaccination. However, for persons who already had a SARS-CoV-2 infection, cytotoxic T-cells climbed to high levels after a single dosage, whereas for those who were naive, cytotoxic T-cells increased only after a second dose several weeks after the original vaccination [32].

Numerous studies have been conducted to investigate the clinical effects of steroids in COVID-19. RECOVERY Collaborative Group was the first randomized clinical research to show that dexamethasone at a dose of 6 mg daily for 10 days improves the 28-day death rate in COVID-19 patients hospitalized and requiring oxygen therapy and/or invasive mechanical breathing [33].

The MEDEAS trial, a recent Italian study, compared the administration of dexamethasone to methylprednisolone in patients hospitalized for COVID-19 pneumonia with respiratory failure. Yet, for more severe patients (P/F < 200), methylprednisolone reduces ICU admission rate and CRP values more than dexamethasone [34].

Van de et al., have looked into the clinical impact of anakinra on COVID-19. A prospective cohort study from the Netherlands found that anakinra therapy reduced clinical symptoms of hyperinflammation in COVID-19 patients after 28 days of treatment [38]. Anakinra has thus been approved for the treatment of COVID-19 individuals with pneumonia who need

supplemental oxygen and are at risk of developing severe respiratory failure. On the contrary, only a few trials have been undertaken to determine the efficacy of canakinumab. Two Italian observational studies revealed that canakinumab medication could have therapeutic benefits in non-ICU patients with mild or severe COVID-19, such as increasing oxygenation [35].

The use of mesenchymal stem cells (MSCs) has been investigated by Taylor et al., for severe COVID-19. MSCs appear to possess immunoregulatory and antiapoptotic properties, in addition to the ability to stimulate angiogenesis and tissue repair. Furthermore, MSCs have been demonstrated to produce angiopoietin 1 and keratinocyte growth factor, which have impacts on alveolar-capillary membrane healing. MSC activity may be particularly beneficial because, following venous injection, they exhibit homing in the lungs and migrate to the most injured tissues [36].

Research problem:

Several biomarkers of cellular immune response, inflammation, and oxidative stress have been employed as severity indicators in COVID-19 patients. Individuals with mild COVID-19 demonstrated productive innate and adaptive immunity, as seen by initial transitory increases in monocytes and NK cells, followed by long-term increases in memory T and B cells. Individuals with severe disease have showed symptoms suggestive of an immune response dysregulation by delayed and extended elevations in Tfh cells, HLA-DRlo monocytes, and activated CD8+ T cells [20-22].

More research is needed to understand the long-term implications of COVID-19 infection caused by SARS-CoV-2. The consequences and clinical evidence of COVID-19 remain unclear, and the pathophysiology has yet to be thoroughly characterized. Studies on the etiology of COVID-19 disease suggest that not all infected people acquire severe respiratory disease [17].

Materials and Methods

Study population the study included a total of 100 participants; 50 patients with positive COVID-19 and 50 healthy controls matched with age, gender, lifestyle and smoking habits.

- **Inclusion Criteria:**
  - Adults aged 18 years and older.

- Confirmed COVID-19 infection (based on PCR or antigen testing).
- Informed consent to participate.

- **Exclusion Criteria:**
  - History of autoimmune disorders.
  - Immunocompromised individuals (e.g., due to medication or underlying conditions).
  - Pregnant or breastfeeding women.

2. Sample Size

Sample size was calculated based on expected differences in immune responses between COVID-19 patients and a control group by using MedCalc software Version 22.009 package for biomedical research. The criteria used for sample size calculation were as follows: Two-sided confidence level 95%, Power 80% and two study groups, ratio is 1:1. Interleukin levels significantly increased in SARS-CoV-2-positive patients when compared with healthy controls as it was 32.82 ± 42.79 in COVID 19 and14.81 ± 13.66 in healthy control according to the results of Basaran et al., 2023. The sample size based on the previously mentioned criteria was found to be 100 participants (50 participants in each group) [38].

Method of data collection:

Before sample collection, a detailed questionnaire about the health status, medical history, and alcohol and smoking habits of patients and controls were obtained. Subjects who reported chronic diseases, radiotherapy or chemotherapy were excluded. No alcohol intake was reported in all the study groups.

Preparation of Samples

Peripheral blood samples of 10 mL were collected, stored at +4 °C, and processed within two hours. Two-milliliter blood samples taken in tubes without anticoagulant were centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum samples were tested for biochemical parameters such as serum C-reactive protein (CRP), ferritin, d-dimer, fibrinogen, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Two-milliliter blood samples were taken in EDTA-containing tubes and centrifuged for 10 minutes at 3000 rpm to separate plasma [39].

Determination of Biochemical Parameters

Hemograms and several biochemical parameters, including CRP, ferritin, d-dimer, fibrinogen, AST, and ALT, were measured using the Sysmex XN-2000 hematology autoanalyzer. Samples were analyzed in duplicate, with results expressed in standard units: WBCs, platelets, and differential counts in $10^3/\mu L$; RBCs in $10^6/\mu L$; hemoglobin and MCHC in g/dL; MCV, RDW, PDW, and MPV in femtoliters; MCH in picograms; hematocrit as a percentage; CRP and fibrinogen in mg/L; ferritin in ng/mL; d-dimer in µg/mL; and AST and ALT in U/dL.

Determination of and Immune Parameters

Serum was obtained from all subjects on hospital day 1, processed, and stored at −80 °C until testing. Individual aliquots were used on the day of testing and analyzed according to the manufacturer's instructions. In brief, samples were allowed to thaw to room temperature, mixed by vortexing, and centrifuged immediately prior to use. Cytokines and chemokines were measured in each sample using a custom Milliplex panel (HCYTA-60K-10; IFN-α2, IFN-γ, IL-1β, IL-2 IL-6, IL-8, IL-10, IP-10/CXCL10, MCP-1/CCL2, TNF-α; MilliporeSigma, Burlington, MA USA). Serum IgG and IgM were detected using the Milliplex MAP SARS-CoV-2 Antigen Panel 1 IgG (HC12SERG-8k5K) and IgM (HC19SERM-85K) against spike subunits (S1 and S2), receptor binding domain (RBD), and nucleocapsid (N) proteins according to the manufacturer's instructions. The Milliplex assays used in this study are intended for research use and were not validated for clinical use. Information from the assay manufacturer indicates intra-assay precision within 15% CV and inter-assay precision within 20% CV.

Statistical Analysis

Data were analyzed using the IBM® SPSS statistical soft-ware, version 27. We used the one-sample Kolmogorov—Smirnov test to check the normality of data and part of data were parametric while another were non parametric. Numerical data was presented as mean and standard deviation (SD) when it was normally distributed while median and interquartile range was used for non-normally distributed data. Categorical data was presented as number and percentage. Chi-squared test was used for comparing the qualitative data. Student t- test was used to compare the

means in different groups. The magnitude of linear relationships was calculated using Pearson correlation analysis. The level of significance was adopted at $p<0.05$.

Results:

Table (1): Demographic data among the study participants

Demographic data	Patients (n =50)		Control (n=50)		Test of sig (p value)
	No.	%	No.	%	
Age Min - max Mean + SD	18 – 75 42.36 + 15.25		19 – 73 45.64 + 16.39		t=1.37 P =0.14
Gender Male Female	 21 29	 42.0% 58.0%	 26 24	 52.0% 48.0%	X^2 =1.47 P =0.21
Current cigarette smoker Yes No	 23 27	 46.0% 54.0%	 25	 50.0% 50.0%	X^2 =1.71 P =0.24
Body mass index Min - max Mean + SD	18.65 – 32.41 26.43 ± 4.52		18.79 – 32.65 25.38 ± 4.65		t=1.05 P =0.42

Table (1) found that there were no significant differences between the cases and controls regarding to the demographic data. The mean age of for cases was 42.36 ± 15.25 years (with range of **18 – 75)** and 45.64 ± 16.39 years for controls. Gender distribution was also comparable, with 42.0% of cases being male and 52.0% of controls. Current cigarette smoking status showed no significant difference either, with 46.0% of cases and 50.0% of controls identified as smokers. The mean value of body mass index (BMI) was 26.43 ± 4.52 for cases and 25.38 ± 4.65 for controls.

Table (2): complete blood count (CBC) analysis of studied groups

		Patients (n =50)	Control (n=50)	p-value
RBCs	Min - Max	3.55–5.94	3.54–5.46	0.65

(10^6/µL)	Mean± SD	4.59 ± 0.87	4.47 ± 0.97	
Hemoglobin gm/dl	Min - Max	8.2-12.6	12.0-15.0	<0.001*
	Mean± SD	9.8 ± 1.0	12.9 ± 1.2	
MCV (Fl)	Min - Max	78.0 - 98.0	74.0-85.0	0.36
	Mean± SD	86.05 ± 3.88	81.0±2.2	
MCH (Pg)	Min - Max	21.0-29.0	23.0-29.0	0.24
	Mean± SD	24.9 ± 2.3	25.4 ± 1.5	
HCT (%)	Min - Max	28.1–48.36	29.5–49.6	0.46
	Mean± SD	42.61 ± 4.65	41.98 ± 3.98	
Platelets (10^3/µL)	Min - Max	140 – 550	164 – 401	<0.001*
	Mean± SD	336.82 ± 120.36	251.76 ± 79.57	
Total leucocyte count /(10^3/µL)	Min - Max	4.20 - 10.0	4.0-8.0	0.431
	Mean± SD	7.3+ 1.35	6.6+ 1.65	
Neutrophils (10^3/µL)	Min - Max	1.89 – 6.48	2.98 – 7.65	0.127
	Mean± SD	4.04 ± 1.68	5.18 ± 2.36	
Lymphocytes (10^3/µL)	Min - Max	0.39 – 1.64	1.87 – 4.68	<0.001*
	Mean± SD	0.78 ± 0.29	2.85 ± 0.98	
Monocytes (10^3/µL)	Min - Max	0.08–1.10	0.10–1.10	0.49
	Mean± SD	0.35 ± 0.26	0.47 ± 0.28	
Eosinophils (10^3/µL)	Min - Max	0.0–0.16	0.0–0.40	<0.001*
	Mean± SD	0.02 ± 0.04	0.18 ± 0.10	
Basophile (10^3/µL)	Min - Max	0.0–0.03	0.01–0.30	0.36
	Mean± SD	0.02 ± 0.01	0.03 ± 0.05	

Table (2) showed the complete blood count (CBC) analysis which revealed significant differences between the COVID -19 cases and healthy controls in some parameters. The mean value of red blood cell counts (RBCs) was 4.59 ± 0.87 × 10^6/µL for COVID-19 and 4.47 ± 0.97 × 10^6/µL for controls with no statistical significant difference in between ($p = 0.65$). However, hemoglobin levels were significantly lower in patients, averaging 9.8 ± 1.0 g/dL compared to 12.9 ± 1.2 g/dL in controls ($p < 0.001$). The mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) showed no significant differences ($p = 0.36$ and $p =$

0.24, respectively). A significant increase in platelet counts was observed in patients, with a mean of 336.82 ± 120.36 × 10^3/µL versus 251.76 ± 79.57 × 10^3/µL in controls ($p < 0.001$). The lymphocyte counts were significantly lower in patients (0.78 ± 0.29 × 10^3/µL) compared to controls (2.85 ± 0.98 × 10^3/µL; $p = 0.001$). Eosinophil counts also demonstrated a significant difference, with patients levels (0.02 ± 0.04 × 10^3/µL) than controls (0.18 ± 0.10 × 10^3/µL; $p = 0.001$).

Table (3): Biochemical parameters of the study groups

		COVID-19 Patients (n =50)	Control (n=50)	p-value
CRP (mg/L)	Min - Max	30.0 – 305.0	0.41– 10.9	**<0.001***
	Mean± SD	124.87 ± 69.84	3.98 ± 4.51	
Ferritin (ng/mL)	Min - Max	67.2–1798	15.01– 254.95	**<0.001***
	Mean± SD	899.5 ± 451.9	69.47 ± 48.8	
d-Dimer (µg/mL)	Min - Max	745 – 4750	69– 465	**<0.001***
	Mean± SD	1478 ± 119.7	272.7 ± 169.7	
Fibrinogen (mg/dL)	Min - Max	74 – 897	68 – 443	**<0.001***
	Mean± SD	486.2 ± 204.7	260.6 ± 82.3	
AST (U/dL)	Min - Max	20 -54	11 – 41	**0.63**
	Mean± SD	19.24 ± 21.41	22.51 ± 7.65	
ALT (U/dL)	Min - Max	15 – 49	13 – 39	**0.65**
	Mean± SD	24.98 ± 7.35	19.70 ± 5.21	

The inflammatory and biochemical markers showed significant differences between COVID-19 patients and controls. C-reactive protein (CRP) levels were markedly elevated in patients, with a mean of 124.87 ± 69.84 mg/L compared to 3.98 ± 4.51 mg/L in controls ($p < 0.001$). Ferritin levels also revealed a significant increase in patients, with a mean of 899.5 ± 451.9 ng/mL, versus 69.47 ± 48.8 ng/mL in controls ($p < 0.001$). D-dimer levels were significantly higher in the patient group, with a mean of 1478 ± 119.7 µg/mL compared to 272.7 ± 169.7 µg/mL in controls ($p < 0.001$). Fibrinogen levels showed a mean of 486.2 ± 204.7 mg/dL in patients, significantly higher than the 260.6 ± 82.3 mg/dL in controls ($p < 0.001$). In contrast, liver enzymes AST and ALT did not found significant differences, with means of 19.24 ± 21.41 U/dL and 24.98 ± 7.35 U/dL for patients, respectively, compared to 22.51 ± 7.65 U/dL and 19.70 ± 5.21 U/dL for controls.

Table (4): immune parameters of the study groups

		COVID-19 Patients (n =50)	**Control (n=50)**	**p-value**
IL-6	Min - Max	10.0 - 214.0	0.9 - 379.2	0.983
	Mean± SD	25.9 + 14.6	27.81 + 20.36	
IL-8	Min - Max	9.8 - 170.4	12.82 – 145.3	0.660
	Mean± SD	39.8 + 20.36	24.61 + 20.65	
IL-10	Min - Max	0.7 - 79.7	0.0 - 156.5	0.359
	Mean± SD	18.6 + 15.66	17.93 + 14.95	
IP-10	Min - Max	(115.8 – 954.36	99.2 - 425.64	0.005*
	Mean± SD	493.3 + 142.32	259.9 + 78.65	
MCP-1	Min - Max	365.32 - 19681.9	(212.6 - 973.7)	0.015*
	Mean± SD	578.94 + 145.32	365.54 + 120.01	
TNF-α	Min - Max	2.2 - 1939.7	2.1–355.87	0.047*
	Mean± SD	143.7+15.65	37.0 +14.52	

The analysis of cytokine levels revealed notable differences between COVID-19 patients and controls. Interleukin-6 (IL-6) levels showed no significant difference, with means of 25.9 ± 14.6 pg/mL for patients and 27.81 ± 20.36 pg/mL for controls. Interleukin-10 (IL-10) levels also did

not differ significantly, with patients showing a mean of 18.6 ± 15.66 pg/mL compared to 17.93 ± 14.95 pg/mL in controls (p = 0.359). In contrast, significant differences were observed in other cytokines. Interferon gamma-induced protein 10 (IP-10) levels were markedly elevated in patients, with a mean of 493.3 ± 142.32 pg/mL versus 259.9 pg/mL in controls (p = 0.005). Similarly, monocyte chemoattractant protein-1 (MCP-1) levels were significantly higher in the patient group, averaging 578.94 ± 145.32 pg/mL compared to 365.54 ± 120.01 pg/mL in controls (p = 0.015). Tumor necrosis factor-alpha (TNF-α) also demonstrated significant elevation in patients, with a mean of 143.7 ± 15.65 pg/mL compared to 37.0 ± 14.52 pg/mL in controls (p = 0.047).

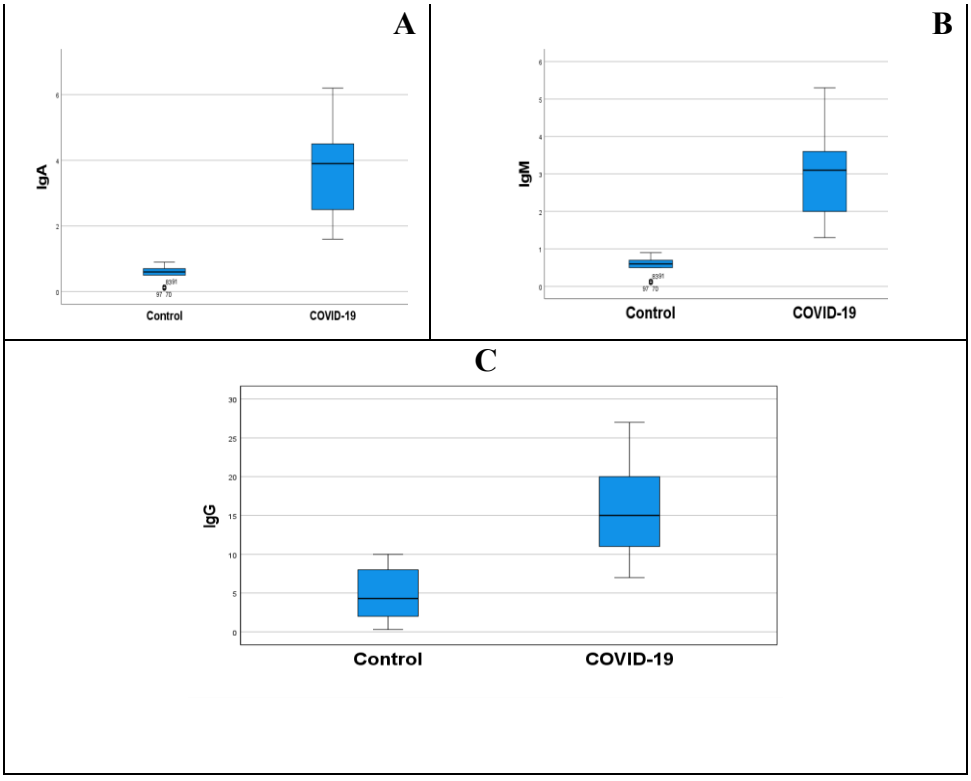

Fig (1): Differences in the serum concentration of IgA (A), IgM (B) and IgG (C) among the groups of COVID-19 patients and control groups (differences significant – Kruskal-Wallis test).

Figure (1) revealed that there was a decreased concentrations of serum IgA (Fig1A), IgM (Fig 1B), but not IgG (Fig 1C) in the Control group

compared to COVID-19 with statistically significant difference (p =0.001,0.041 and 0.002* respectively).

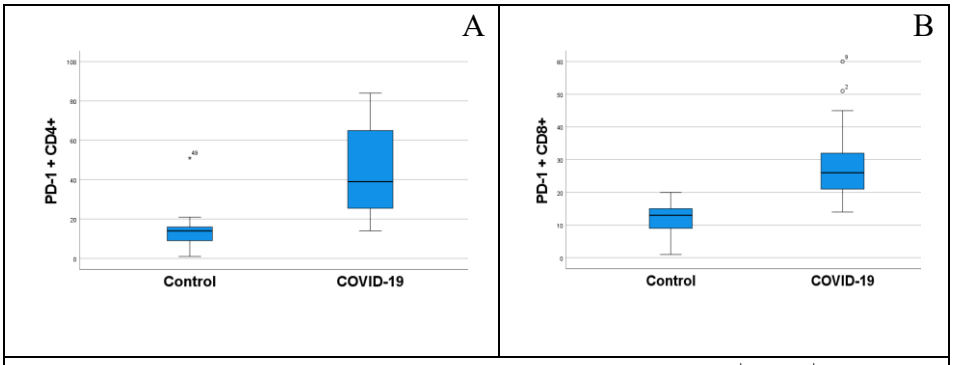

Fig (2): The differences in expression of PD-1 on $CD3^{+}CD4^{+}$ (A; p = 0.001*) and $CD3^{+}CD8^{+}$ (B; p = 0.041*)

Figure (2) revealed that there was a decreased concentrations of serum PD-1 on CD3+CD4+ (fig 2A) and CD3+CD8+ (fig 2B) in the Control group compared to COVID-19 with statistically significant difference (p = 0.001* and 0.041*respectively).

Discussion

The novel coronavirus 2019 (COVID-19) infection, which has put many people's health at risk in a number of countries, is the century's most serious epidemic. It is thought to have a significant role in SARS-CoV infection (39, 40). Because there are a few clinical investigations examining the function of immunological indicators in COVID-19 pathogenesis, we sought to evaluate the effects of COVID-19 on certain critical biochemical and immune biomarkers in COVID-19-positive patients [17].

COVID-19 infection can trigger a powerful immunological response, including innate immune activation and antiviral immunity. However, the shift between innate and adaptive immune responses is important to determining the clinical course and prognosis of COVID-19 infection [18]. Early immune responses to COVID-19 are generally protective in viral clearance, however exaggerated and dysregulated immune responses, often described as the "cytokine storm," can induce harm to tissues, contributing to poor clinical outcomes [20].

The present study found that patients with COVID-19 symptoms had higher levels of CRP, ferritin, d-dimer, and lower levels of lymphocytes

and eosinophils, which is in agreement with the findings of Martins-Filho et al., who found higher levels of IL-6, CRP, and ferritin, as well as increased coagulation abnormalities such as prolonged prothrombin time, increased d-dimer, and thrombocytopenia. They hypothesized that these alterations are key predictors of COVID-19 mortality. Aside from increased levels of CRP, ferritin, d-dimer, and fibrinogen, we discovered greater levels of AST in patients with COVID-19 symptoms than in healthy controls [41]. Çakırca et al., found that COVID-19-positive patients in the intensive care unit had greater levels of white blood cells, neutrophils, CRP, procalcitonin, ferritin, fibrinogen, and urea, but lower levels of lymphocytes and albumin compared to non-ICU patients [42].

In a prospective cohort study of 120 COVID-19-positive patients and 60 healthy controls, neutrophils were shown to be greater in the critical group, whereas lymphocytes were lower in the severe and critical groups compared to the mild group. Severe and critical cases had greater CRP, ferritin, and d-dimer levels than mild COVID-19 cases [43].

Lymphopenia is a significant laboratory finding in COVID-19 patients, with both diagnostic and prognostic implications. Dysregulated immunological responses contribute to COVID-19 pathogenesis [44]. Our findings show considerable progressive lymphopenia, in accordance with previously published data [45-48]. Furthermore, the majority of COVID - 19 cases we treated showed eosinopenia. A meta-analysis of 1289 COVID-19 cases discovered a strong link between raised leucocyte and lowered lymphocyte counts in relation to disease severity. In published investigations, the majority of severe cases reported on admission with a total lymphocyte count $<1 \times 109/L$, while non-severe cases had lymphocytes above this threshold [49].

It is unclear what beneath the phenomenon that causes lymphopenia in COVID-19 patients, several authors speculate on increased apoptosis, inhibition or down-regulation of lymphocytes induced by cytokines, metabolic disorders (e.g., lactate acidosis), and increased glucocorticoids. It has also been hypothesized that viruses can directly infect and destroy lymphocytes and lymphatic tissue. Whereas lymphopenia is an essential laboratory marker of SARS-CoV-2 infection, medical professionals need to be understood that it is not limited to COVID-19 and may happen in

other viral pneumonias as well. Eosinopenia, while related with COVID-19, is not exclusive to this disease and has been reported in other viral disorders [50].

An overreactive immune response produces excessive pro-inflammatory cytokines and chemokines, which have been widely characterized [33]. Of these enhanced pro-inflammatory cytokines, IL-6 is the most studied and is a primary driver of cytokine dysregulation, which is responsible for hyper-inflammation in the lungs in COVID-19 patients [42]. Only IL-6 is commercially accessible as a single marker test for diagnosis usage according to an FDA Emergency usage Authorization in the context of the present COVID-19 pandemic. Its purpose is to help detect severe inflammatory responses among individuals with confirmed COVID-19 illness, when combined with clinical observations and the results of other laboratory tests.

Herold et al. found that the maximal IL-6 level was highly linked with the need for mechanical breathing in patients hospitalized with COVID-19, supporting its emergency use authorization [49].

Unexpectedly, the information we provide revealed no significant variations in IL-6 levels between the patient and control groups, with IP-10 being the only cytokine to show significance between COVID-19 patients as well as controls. The results we obtained are consistent with those of Yang et al., who discovered that IP-10 but not IL-6 could have predicted the progression of COVID-19 [51].

IP-10 levels have been found to rise in people infected with a variety of respiratory viruses, and it was recommended as a biomarker for virus detection. While IP-10 is not presently accessible in healthcare settings, it is part for the recently-cleared MeMed BV test, which is a computerized semi-quantitative an immunoassay that concurrently determines TNF-related apoptosis-inducing ligand (TRAIL), IP-10, and CRP in samples of serum leading to aid in differentiating bacterial from viral infections [50].

The current study observed a significantly greater percentage of CD4+CD8+ cells in COVID-19 cases. Jiang Y. et al. (2020) found a higher percentage of CD38+CD8+ cells and HLA-DR+CD8+ cells in COVID-19 patients compared to healthy controls, but no significant differences were found among different disease severity groups [51].

Wang F. et al. (2020) found that HLA-DR expression was considerably higher on both CD3+CD4+ and CD3+CD8+ cells in severe and extremely severe patients than in moderate instances. Increased activity of CD3+CD4+ and CD3+CD8+ cells caused by continuous and excess inflammatory responses could result in the occurrence of more severe disease in SARS-CoV-2 infected patients, eventually leading to cell fatigue in later stages of the disease [52].

The study's strengths involve an integrated investigation of cytokine and immunological response (antibody) indicators. However, we recognized a few limitations. First, given the small sample size of the research we conducted and the fact that our individuals were enrolled after widespread vaccination towards COVID-19, this may be weakened or disguised in a vaccinated population. Second, because our investigation used retrospective data passively collection, several critical clinical factors, such as severity information for COVID-negative control patients, hadn't been accessible. Third, because the patient group was enrolled in the late stages of the pandemic and they received antiviral medicines and antibody treatment that are readily accessible to patients hospitalized with more severe forms of COVID-19. These therapies may reduce the effects on biomarker.

Conclusion:

The immune system plays an important role in slowing the progression of COVID-19 to more serious stages of the illness. SARS-CoV-2 infection activates both innate and adaptive immune responses. The development of immune-modulating medicines that seek to stop or regulate the cytokine appears to be the most effective available weapons against COVID-19.

The immune system and changes in its reactivity have an obvious role in several areas of COVID-19 pathology, ranging from a greater vulnerability to infection overall to modification of the clinical manifestations and deciding the disease's fate. Another intriguing topic is transitory immune depletion, which is measured by measuring various surface markers on immune cells. The loss of expression of these markers may serve not only as a predictive tool, but also as a possible therapeutic target, particularly in the early stages of COVID-19. Based on our findings, we discovered a novel opportunity for immunological markers to track illness progression

and establish a prognosis.

Conflict of Interest

The authors state that the study was done without any kind of commercial or financial links that could be seen as a potential conflict of interest.

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