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Assessment of Inflammatory Markers and Clinicopathological Characteristics of COVID-19

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Abstract: The objective of this cross-sectional study was to find out the correlation of severity of the COVID-19 with sex, age, and inflammatory markers at the time of admission. The study was conducted at Ziauddin Hospital North Nazimabad. 264 PCR positive for COVID-19 patients were included in this study, and the data were retrieved from the hospital records after taking the institutional ERC approval. This study will help better understand the risk factors and pathogenesis of the disease, interpret its severity based on signs, symptoms, and clinicopathological parameters. So, we can classify the patients, and better specific treatment options can be offered. The patients were divided into =<50 years and > 50 years to see the link between age and severity. In 103 severe cases, the age was greater than 50 years, and the p-value was 0.001. Males were more afflicted by severe disease (91) than females (36), but there was no statistically significant relationship between severity and gender (p-value of 0.695). Ferritin levels were found to be greater in 199 (75.4%) patients (Mean 1360, Range 5 to 36795), and lactate dehydrogenase (LDH) levels were found to be higher in 214 (81.4%) patients (545.69, Range 106 to 13190). Higher WBC numbers (Mean 10.89, Range 1.2 to 43.1) and high neutrophil counts (Mean 81.1, Range 45 to 96) were found in 89.7% of the people, while high lymphocyte counts were seen in 8.1 percent (Mean 12.7, Range 2 to 48). CRP and D-dimers have a statistically significant relationship with COVID-19 severity. Furthermore, we aimed to establish which inflammatory marker of COVID-19 is more peculiar and sensitive and found that ferritin and LDH are both equally sensitive (51.3%), with LDH being more specific (60%) than others in predicting the severity of COVID-19.

Keywords: COVID-19, pandemic, cytokine storm, C-reactive protein, lactate dehydrogenase, ferritin, angiotensin-converting enzyme 2, transmembrane protease, serine 2; lymphocytes.

新冠肺炎炎症标志物和临床病理特征的评估

摘要：这项横断面研究的目的是找出入院时新冠肺炎的严重程度与性别、年龄和炎症标志物的相关性。该研究是在齐奥丁医院北纳兹马巴德进行的。这项研究包括了264名新冠肺炎患者的聚合酶链反应阳性，数据是在获得机构电子资源委员会批准后从医院记录中检索的。这项研究将有助于更好地了解该疾病的危险因素和发病机制，根据体征、症状和临床病理参数解释其严重程度。因此，我们可以对患者进行分类，并可以提供更好的具体治疗方案。将患者分为 = <50 岁和 > 50 岁以查看年龄与严重程度之间的联系。在 103 例重症病例中，年龄大于 50 岁，p 值为 0.001。男性比女性 (36) 更容易受到严重疾病的折磨 (91)，但严重程度和性别之间没有统计学上的显着关系（p 值为 0.695）。发现 199 (75.4%) 名患者的铁蛋白水平较高（平均 1360，范围 5 至 36795），发现 214 名（81.4%）患者（545.69，范围 106 至 13190）的乳酸脱氢酶

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(乳酸脱氢酶) 水平较高）。在 89.7% 的人中发现了较高的白细胞数量（平均 10.89，范围 1.2 到 43.1）和高中性粒细胞计数（平均 81.1，范围 45 到 96），而在 8.1%（平均 12.7，范围 2 到 96）中发现高淋巴细胞计数48)。CRP 和 D-二聚体与新冠肺炎的严重程度具有统计学上的显着关系。此外，我们旨在确定新冠肺炎的哪个炎症标志物更奇特和更敏感，并发现铁蛋白和乳酸脱氢酶都同样敏感(51.3%)，乳酸脱氢酶在预测冠状病毒病的严重程度方面比其他人更具体 (60%) -19.

关键词：新冠肺炎、大流行、细胞因子风暴、C-反应蛋白、乳酸脱氢酶、铁蛋白、血管紧张素转换酶2、跨膜蛋白酶、丝氨酸2；淋巴细胞。

1. Introduction

The world is at war, with the battle being fought between the human race and a newly-emerged novel coronavirus that the World Health Organization (WHO) named Coronavirus Disease 2019 (COVID-19). The magnitude of the problem has increased rapidly since the pandemic first appeared in Wuhan in the West District of Southern China. It has involved more than 200 countries around the globe, affecting around 255 million people, with more than 5 million deaths reported. According to the WHO, 6720771 cases have been reported in South East Asia and 5662558 cases in Europe [1]. Recent updates from Pakistan indicate that more than 293,000 cases have been reported in the country, consisting of more than 128,000 cases in Sindh, 96,000 in Punjab, 35,000 in Khyber Pakhtunkhwa, and 12,000 in Baluchistan, with a total of 6,457 (2.1%) deaths reported [2].

According to phylogenetic studies on the novel virus genome, it is similar to a group of severe acute respiratory syndrome (SARS) coronaviruses that were derived from bats in China. It is not the first pandemic to be caused by a coronavirus, and they have been responsible for three epidemics over the last two decades. These include SARS and the Middle East respiratory syndrome (MERS) in addition to COVID-19 [3].

The coronaviruses family of viruses cause respiratory infections. They were first isolated in 1937 and named coronaviruses in 1965 because, microscopically, they have a crown-like appearance. To date, the following varieties of coronaviruses have been identified: the alpha coronaviruses, HCoV-229E and HCoV-NL63; the beta coronaviruses, HCoV-OC43 and HCoV-HKU1; SARS-CoV, which causes SARS; MERS-CoV, which causes the Middle East respiratory syndrome (MERS); and SARS-CoV-2, the new coronavirus identified in China at the end of 2019, which has resulted in the disease known as COVID-19 [4].

The coronavirus is a positive-sense, enveloped RNA genome virus, which has protein spikes on its surface called S-protein. The coronavirus contains four important pathogenic structural proteins that are very important for its immunogenicity and pathogenesis. These are the spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. These four proteins are the primary agents that enable the coronavirus to bind to its receptors in human cells [5]. Many studies have confirmed that SARS-CoV needs ACE2 receptors for its entry into human cells, and this applies equally to COVID-19. These ACE2 receptors are encoded by the ACE2 gene, located on the X-Chromosome. The binding of the coronavirus with the host cell's ACE2 receptor is the main requirement for its pathogenesis.

The coronavirus binds to the ACE2 receptors by means of the receptor-binding region of the S-protein. Once the virus binds to its receptors, it creates a protein receptor complex that is endocytosed by the host cells. Here TMPRSS 2, a trans membrane and surface serine protease with proteolytic activity, cleaves the complex and releases the S-protein into the cytoplasm. Following the release of the S-protein, the viral genome begins to replicate [6]. Once the viral entry has occurred, viral antigens are presented to the antigen presenting cells (APC) in combination with a major histocompatibility complex that is an integral part of the body's anti-viral activity. Here, the virus particles are recognized by virus-specific cytotoxic T lymphocytes (CTLs). Subsequently, humoral and cellular immunity is stimulated by antigen presentation, which is mediated by virus-specific B and T cells. The coronavirus has the same pattern of IgM and IgG antibody production as other common viral infections in which the IgM disappears from the body by the end of the 12th week, while the IgG persists for a longer period of time, showing its protective effect through the formation of memory cells [7]. In addition to the production of antibodies, these virus-specific B and T cells are also responsible for the release of large amounts of pro-inflammatory cytokines, such as Interleukin-6 (IL-6), Interleukin-8 (IL-8), Interleukin-12 (IL-12), Interleukin-33 (IL-33), INF-α, INF-γ, and TGF-β and for chemokines, such as CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10, and many more.

This release is known as a “Cytokine Storm,” during which the immune system triggers a violent attack on the body, leading to shock, acute respiratory distress syndrome (ARDS), thromboembolism, multi-organ failure, and in some cases, death [8].

Some individuals with SARS-CoV-2 infection remain asymptomatic, while in others, the infection causes mild to severe COVID-19 disease or COVID-19 pneumonia, requiring intensive care support. In some instances, the disease results in death, especially in older adults. Persons affected by coronavirus can have a series of clinical manifestations, including, cough, fever, headache, myalgia, fatigue, dyspnea, even to the extent of acute respiratory distress syndrome, acute cardiac injury, and secondary infections [9]. In addition to these common symptoms, patients can develop intestinal symptoms (e.g., vomiting, diarrhea). Diarrhea was experienced by around 20% to 25% of patients infected with MERS-CoV or SARS-CoVA, with many infected people being admitted to ICU critical care units due to the severity of their symptoms. Radiological findings, such as lung opacities, ground glass appearance, and bilateral infiltrates are important findings associated with serious COVID-19 illness. Most of the patients also show changes in their inflammatory markers, such as an increased cell count of D-dimer, C-reactive protein (CRP), ferroprotein, erythrocyte sedimentation rate (ESR), LDH and others, such as urea, and creatinine [10]. Therefore, in this study we correlated the severity of the COVID-19 with sex, age, and inflammatory markers at the time of admission.

2. Methods

This is a cross-sectional study conducted on data acquired from the medical records of patients admitted to the Dr. Ziauddin Hospital, North Nazimabad, Karachi. First, approval was obtained from the Ethical Review Committee of the institution (Reference code: 2650920SKPAT). Data was collected with the explicit permission of the hospital. Patients were included in the study if they had a positive reverse transcriptase polymerase chain reaction (RT-PCR) test for SARS-CoV-2 before or during admission to the Dr. Ziauddin Hospital, North Nazimabad Campus, and including the ICU and HDU wards, during the period April 2020 to June 2020. A total of 264 patients were included in the study. Laboratory values were taken from blood tests performed as part of routine patient care (for CBC, CRP, procalcitonin, ProBnp, ferritin, LDH, urea, creatinine, electrolytes, and others). Values were taken from the first time a particular test was performed during the course of admission. Co-morbidities were recorded using data from medical records, and these were categorized as diabetes mellitus, hypertension, or other. The severity of COVID-19 was defined according to the WHO guidelines and using the clinical signs and symptoms recorded on the medical records, including the findings of chest radiographs, and the oxygenation status of the patient.

According to the COVID-19 diagnosis and treatment recommendations, all patients were diagnosed and classified into mild, moderate, severe, and critical types based on the severity of their symptoms. At least one of the following criteria should be met by severe patients: Shortness of breath is the first symptom, with a respiration rate (RR) of 30 times per minute. Second, in a resting state, oxygen saturation is 93 percent. Third, a ratio of 300 mm Hg between the partial pressure of arterial oxygen (PaO2) and the fraction of inspired oxygen (FiO2). Severe cases were defined as lesions that progressed by more than 50% on pulmonary imaging in less than 24 hours. When one of the following conditions was met, a critical case was defined: First, there is respiratory failure, which necessitates mechanical ventilation. Then, there was the shock. Third, it is treated in an intensive care unit with other organ failures.

Data were expressed as mean ± SD, median (interquartile range [IQR]], or percentages as appropriate. To compare the differences between the two groups, mean values and percentages were used between the two groups by the Student t-test and chi-square ($\chi 2$) test. Statistical analyses were performed using SPSS software (Version 22). $P < 0.05$ (two-tailed) was considered statistically significant.

2.1. Statistical Analysis

Statistical analysis was performed using SPSS Version 21. For qualitative variables, ratio and percentages were used, and for quantitative variables, mean and standard deviation were used. The relationship between B7-H4 expression and clinicopathological factors such age, tumor grade, and stage was investigated using the chi-square test. A P-value of 0.05 was established as the statistical significance criterion.

3. Results

The objective of the current study was to associate the clinicopathological parameters of COVID-19 with severity of the disease. Current research also relates the signs and symptoms of COVID-19 with severity. This study has found the more sensitive and specific inflammatory markers related to the disease. Majority of patients included in this study were male (189, 71.6%), while 75 (28.4%) were female. The mean age of participants was 56.42 ± 14.89 (range of 16 to 87 years). A total of 127 (48.1%) showed signs and symptoms of severe disease and were admitted in the ICU, while remaining had mild (102, 38.6%) to moderate disease (35, 13.3%). We divided the patients into two age groups (≤ 50 years and > 50 years) to find the association between age and severity and found a highly significant association, as 103 of the severe cases were more than 50 years old, with a p-value of

0.001. Males were affected more with severe disease (91) as compared with females (36), showing an insignificant association between severity and gender (p-value of 0.695).

Table 1 Association of sex and age with the severity of COVID-19

Parameter		Severity			P-Value
		Mild	Moderate	Severe	
Gender	Female	71	27	91	
	Male	31	8	36	0.695
Age	<=50	43	10	24	
	>50	59	25	103	0.001

The patients had varied symptoms of fever (230, 87.1%), cough (152, 57.6%), SOB (191, 72.3%), body ache (91, 34.5%), and other systemic complaints like diarrhea, nausea, vomiting, vertigo, insomnia, anxiety, and many more. Blood inflammatory markers were taken at the time of admission as the routine test showed higher CRP levels in 256 (97%) while 8 (3%) had normal levels (Mean 145.38, Range 0.13 to 792.38). Also, the pro-calcitonin levels were higher in 255 (96.6%) patients, and the remaining 9 (3.4%) had normal levels (Mean 1.50, Range 0.02 to 53.87). D-dimer levels were higher in 114 (43.2%) with normal levels in 150 (56.8%) patients (Mean 1811.55, Range 122 to 15000). Ferritin was found to be higher in 199 (75.4%) individuals (Mean 1360, Range 5 to 36795), and LDH was raised in 214 (81.4%) patients (545.69, Range 106 to 13190). 89.7% of the individuals have higher WBC counts (Mean 10.89, Range 1.2 to 43.1) with high neutrophil counts (Mean 81.1, Range 45 to 96), while 8.1% showed high lymphocyte counts (Mean 12.7, Range 2 to 48). CRP and D-dimers have highly significant statistical links with the severity of COVID-19 (Table 2).

Table 2 Frequency of inflammatory blood markers and statistical estimates with the severity of COVID-19

Inflammatory Marker	Severity	Normal	High	Mean ± SD	P-Value
CRP ≥ 0.8 mg/dL	Mild	7	95	145.38 ± 119.9	0.011
	Moderate	1	34		
	Severe	0	127		
D-dimer ≥ 0.50 µg/mL	Mild	69	33	1811.55 ± 2188.15	0.002
	Moderate	23	12		
	Severe	58	69		
Pro-calcitonin ≥ 0.5 ng/mL	Mild	6	96	1.5 ± 4.93	0.078
	Moderate	2	33		
	Severe	1	126		
Ferritin ≥ 337 ng/mL	Mild	14	88	1360 ± 3088.81	0.115
	Moderate	10	24		
	Severe	25	102		
LDH ≥ 280 U/L	Mild	9	93	545.96 ± 928.18	0.209
	Moderate	1	34		
	Severe	16	111		

Furthermore we also sought to find out the more specific and sensitive inflammatory marker of COVID-19 where we determined that ferritin and LDH are equally sensitive (51.3%) while LDH is more specific (60%) compared to others to predict the severity of COVID-19 (Table 3 and Fig. 1).

Table 3 Sensitivity and specificity of inflammatory markers

Inflammatory Marker	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
High Procalcitinin	50.5% (44%–56.8%)	11.1% (.28% – 48%)	94% (92.5% – 95.4%)	0.79% (.12%–4.8%)	49.2% (43% – 55%)
High Dedimer	39.4% (30%–49%)	38.6% (31% – 47%)	32.8% (27% – 38%)	45.6% (39% – 52%)	39% (33% – 45%)
High Ferritin	51.3% (44% – 58%)	53.8% (37% – 70%)	84.6% (79% – 88%)	18.2% (14% – 23%)	51.7% (45% – 58%)
High LDH	51.3% (44%–58%)	60% (32%–83%)	94.9% (90% – 97%)	7.8% (5.2% – 11%)	51.9% (45% – 58%)

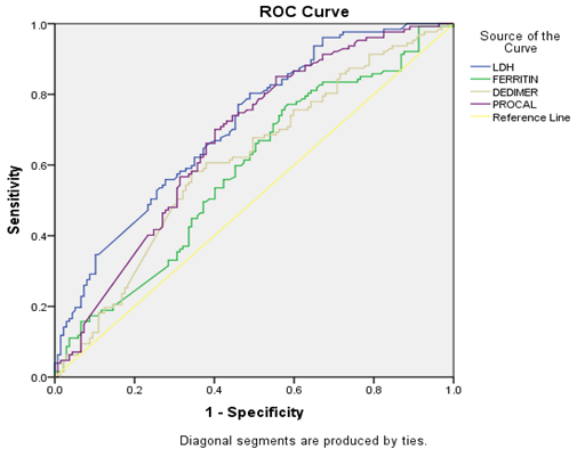

Fig. 1 ROC curve for inflammatory markers

4. Discussion

Coronavirus is a large family of viruses that cause illnesses ranging from the common cold to severe pneumonia, such as SARS and Middle East Respiratory Syndrome (MERS). However, little data on the prognostic factors of COVID-19 have been reported. The number of men is 2.4 times that of women in the deceased patients. While men and women had the same susceptibility, men were more prone to dying. Our analysis found male dominance in the COVID-19 morbidity, which can be explained by the ACE2 gene's location on the X-Chromosome and the TMPRSS2 gene's being involved in prostate cancer. For these reasons, it has been postulated that COVID-19 is more frequent in males than females [12]. A recent case-control study on the Chinese population showed a negative correlation between the ACE2 expression levels and estrogen levels, proving that estrogen has some role in the down-regulation of the ACE2 expression [13]. This might be a protective factor for females to have COVID-19 infection compared to males. Many studies revealed that COVID-19 is more severe in old age groups, so we found in our analysis that a huge number of patients facing the COVID-19 severity are more than 50 years [14].

A recent study correlated the circulating levels of angiotensin-converting enzyme 2 (ACE2) with the susceptibility and severity of COVID-19 and found a higher level of circulating ACE2 for severe cases in comparison with mild to moderate cases. On the other hand, the number of ACE2 receptors decreases in all

tissues in elder persons who are at a greater risk of severe illness; however, its cause is yet to be defined. It has also been noted that the immune system weakens with age, and other comorbidities like DM, hypertension, and diabetes have a huge impact on the weakening of the immune system, which increases their risk of acquiring the COVID-19 infection. Human pathogenic coronaviruses (coronavirus [SARS-CoV] and SARS-CoV-2 extreme acute respiratory syndrome) bind to their target cells via ACE2 that is expressed in the lung, intestinal, kidney, and blood vessel epithelial cells. In patients with type 1 or type 2 diabetes who are treated with ACE inhibitors and angiotensin II type I receptor blockers, ACE2 expression is significantly increased (ARBs). Hypertension is also treated with ACE inhibitors and with ARBs, resulting in ACE22 up regulation. These data indicate that in diabetes, ACE2 expression is increased, and treatment with ACE inhibitors and ARBs improves expression of ACE2. The increased expression of ACE2 would also promote COVID-19 infection. ACE2 polymorphisms, especially in Asian populations, have been related to diabetes mellitus, cerebral stroke, and hypertension. A combination of both therapy and ACE2 polymorphism might result in an individual's sensitivity.

Apart from the ACE2 and TMPRSS2, certain other important gene variants have also been identified for the varied presentation of the disease and are more importantly related to the severity of the disease. These include CXC families, TLR7, ApoE, IFITM3, IFNAR2, TYK2, OAS1 LZTFL1, SLC6A20, DPP9, and CCR2. A genome-wide association study [11] shows that extreme COVID-19 is associated with variants of five primary genes (IFNAR2, TYK2, OAS1, DPP9, and CCR2) that are responsible for antiviral immunity and lung inflammation. However, we have limited knowledge about the genetic makeup of our population and its relation with the COVID-19 severity, so there is an urge for a large population-based study to associate the different variants of important genes involved in the disease. This may help understand the varied manifestation of COVID-19 effect on patients in different populations.

Some researchers now evaluate the relationship between the ABO blood grouping and the COVID-19 susceptibility and severity. Although blood types are genetically inherited, environmental factors can potentially influence which blood types in a population will be passed on more frequently to the next generation. The susceptibility to viral infection is related to the ABO blood group. Different studies revealed that the blood groups of ABO showed different risk associations for SARS-CoV-2 infection resulting in COVID-19. Specifically, an increased risk was associated with blood group A, while a reduced risk was associated with blood group O. Given the similarity of the nucleic acid sequence and the receptor angiotensin-converting enzyme 2 (ACE2) binding, the similarity between SARS-CoV and SARS-CoV-2, lower blood group O susceptibility and higher blood group A susceptibility to COVID-19 may be correlated with the presence of natural anti-blood group antibodies in the blood, particularly anti-A antibodies. Direct studies would be needed to prove this hypothesis.

Some studies also assessed the inflammatory markers associated with the disease like LDH, ferritin levels, CRP, Procalcitonin, D-dimer, and acute phase response proteins. A rise in the levels of inflammatory markers corresponds to the severity and associated risk factors of COVID-19. However, the role of inflammatory markers in monitoring the severity of COVID-19 is still controversial [15]. In our analysis we found varying levels of all inflammatory markers depending on the course of the disease and associated risk factors. CRP is an extremely responsive systemic acute-phase response marker for inflammation, infection, and tissue damage, which could be used as an indicator of inflammation [16]. Many studies report CRP as a strong predictor of COVID-19 because its level increases with the severity of the disease. This is in line with our analysis [17]. The rise in inflammatory markers seen with extreme COVID-19 is reminiscent of increases in related markers during infection with other diseases. For instance, platelet crit (PCT) and ferritin are released into the bloodstream during bacterial infection, and elevated levels in peripheral blood correlate with the severity of infection. The sequence homologies between PCT and ferritin and other human cytokines, such as the tumor necrosis factor (TNF) family, IL-6, etc., support the hypothesis that PCT and ferritin are mediators of inflammation [18].

We also found higher values of serum ferritin in patients with severe COVID-19 disease and those admitted to ICU. In most cases, particularly in severely ill patients, the D-dimer concentration was increased. This indicates a hypercoagulable state and secondary hyperfibrinolysis [19]. Further work is needed to correlate the inflammatory markers with the patients' comorbidities and other medical history, so we could not include these parameters in the current study. In addition, follow-up studies of patients using serial or daily monitoring of blood inflammatory markers needs to be done in cohorts [20].

In routine clinical practice, infection from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is determined by reverse-transcription PCR (RT-PCR), which fails to distinguish between viable, infectious virus and noninfectious viral particles. In the current pandemic, there is growing demand for a more rapid method with higher throughput [21]. Additionally, there have been concerns that patients strongly depend on readily available, accurate, and reliable RT-PCR assays to detect the genome of the

causative agent of SARS-CoV-2 in biological samples [22].

5. Conclusion

COVID-19 continues to be a major health concern and was the predominant setback in the year 2020. Its effects on both mental and physical stress levels were global. Especially affected were the health professionals who worked continuously to combat the deadly infection, many of them dying while working in hospitals to keep others alive. In addition, due to the absence of particular laboratory tests, they faced significant problems in the diagnosis of COVID-19. There is also a vital need for accurate interpretation of laboratory results to enable the correct management of the disease. In our analysis we found that COVID-19 has no sex predilection. However, many studies claim that COVID-19 is more common and more severe in males than in females. Although we did see a larger number of affected males than females, the difference was not statistically significant. It has also been found that older individuals are more severely affected by COVID-19 than younger ones. Varying levels of common inflammatory markers are raised with the severity of COVID-19.

Investigation of the susceptibility to this deadly infection with global involvement is a dire need of time. The varied clinical presentation supports that most of the patients have severe symptoms, and some do not, even with the exposure to the same risk factors, which raises the possibility of some genetic predispositions of individuals. Furthermore, the correlation between the inflammatory markers and clinical disease severity has not been assessed in our population with different genetic structures. Therefore, this study analyzed inflammatory blood markers including CBC, LDH, Ferritin, CRP, D-dimers, and procalcitonin among mild, severe, and critical patients for disease elucidation. The debates are still open to solve many COVID-19 related mysteries and queries that will help clinicians and health providers deal with the pandemic. The current study could be a good addition to the literature updates available nationally and internationally.

6. Limitation

The study's drawbacks include the lack of information on the history of symptoms prior to acute COVID-19 disease and the lack of information on the severity of symptoms. Information regarding the symptoms and laboratory investigations was only obtained at the time of presentation in the hospital. In addition, this is a single-center study with a relatively small number of patients and, for other reasons, with no control group of patients discharged. Researchers have concentrated on the acute phase of COVID-19, but continued monitoring for long-lasting effects after discharge is important. So, follow-up studies are required to evaluate the series of signs, symptoms, laboratory investigations, and outcomes of the current pandemic.

Ethical Approval

IRB: Ethical approval was taken from the Ethical Review Committee (Reference code: 2650920SKPAT).

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