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Can Hypoxia-Inducible Factor 1α be used as a Biomarker to Evaluate Disease Severity and Prognosis in COVID-19 Patients?

COVID-19 Hastalarında Hypoxia-Inducible Factor 1α Hastalık Şiddeti ve Prognozunu Belirlemede Bir Biyomarker Olabilir Mi?

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Abstract

Background: This study was aimed to answer the questions of whether the serum levels of Hypoxia-Inducible Factor 1α (HIF-1α), which is increased by up to 100 times in many tissues including pulmonary tissue in cases of acute lung injury, could be used as a parameter for monitoring the severity and prognosis in COVID-19 patients.

Material and Method: 40 patients, who were admitted to the hospital with COVID-19 clinical symptoms, and 20 healthy control subjects were included in the study. The diagnosis of 20 patients within the patient group were confirmed by the PCR test. The remaining 20 patients were regarded as COVID-19 suspect group. Clinical and laboratory data of patients on admission were recorded. Clinical laboratory tests and serum HIF-1α levels were measured from the blood samples of COVID-19 group on the day of admission and one week after hospitalization. COVID-19 group was divided into four subgroups according to disease severity and HIF-1α values of each group were compared.

Results: In this study, serum HIF-1α values of confirmed COVID-19 patient group were measured higher than healthy control group's serum HIF-1α values, however no significant difference was found for the COVID-19 suspect group. Within confirmed COVID-19 group, serum HIF-1α values on admission were higher than values after hospitalization, whereas Monocyte count, Platelet count and Ferritin values were lower. Among the confirmed COVID-19 cases, critically ill subgroup's serum HIF-1α levels of the first week were significantly lower than mild subgroup's serum HIF-1α levels of both the first week and the day of admission. HIF-1α values of COVID-19 group were strongly negative correlated with age, whereas weakly positive correlated with platelet counts.

Conclusions: HIF-1α, which are thought to prevent alveolar damage, increased in COVID-19 patients. Additionally, low levels of HIF-1α in COVID-19 patients might be considered as a factor responsible for the aggravation of the clinical severity.

Keywords: Biomarker, SARS-CoV-2, hypoxia-inducible factor 1α

Öz

Amaç: Bu çalışmada akut akciğer hasarı durumlarında birçok dokuda ve pulmoner dokuda düzeyleri 100 kat artan HIF -1α'nın serum düzeylerinin COVID-19 hastalarında hastalığın şiddeti ve prognozunu takipte etkili bir parametre olarak kullanılıp kullanılamayacağı sorularına yanıt aramak amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya COVID-19 klinik şikayetleri ile başvuran 40 hasta ve 20 sağlıklı kontrol bireyi dahil edildi. Hasta grubundan 20 hastanın PCR testi ile COVID-19 tanısı doğrulandı. Diğer 20 hasta ise COVID-19 şüpheli grup olarak kabul edildi. Hasta gruplarının başvuru klinik ve laboratuvar verileri kaydedildi. COVID-19 grubu hastaların başvuru günü ve başvuru sonrası 1. hafta kan örneklerinden laboratuvar testleri ve serum HIF-1α düzeyleri ölçüldü. COVID-19 grubu hastalık şiddetine göre dört alt gruba ayrılıp her grubun HIF-1α değerleri birbirleri ile karşılaştırıldı.

Bulgular: Bu çalışmada COVID-19 doğrulanmış hasta grubunda serum HIF-1α değerlerinin sağlıklı kontrol grup serum HIF-1α değerlerinden daha yüksek olduğu bulundu. Bununla birlikte COVID-19 şüpheli grup ile arasında anlamlı farklılık bulunamadı. COVID-19 doğrulanmış hasta grubunda hastane yatış günü serum HIF-1α değerlerinin yatış sonrası HIF-1α değerlerinden yüksek bulunurken, monosit, platelet ve ferritin değerleri ise düşüktü. COVID-19 doğrulanmış hasta grubunun kritik alt grubunun 1. hafta serum HIF-1α değerleri hafif alt grup 0. Gün ve 1. hafta değerlerinden anlamlı düzeyde düşüktü. COVID-19 grup HIF-1α değerleri hasta yaşı ile güçlü negatif korele bulunurken, platelet sayıları ile zayıf pozitif korele bulundu.

Sonuç: COVID-19 hastalarında alveolar hasarı engellediği düşünülen HIF-1α düzeyleri yükselmektedir. Bununla birlikte HIF-1α düşük seyreden COVID-19 hastalarında yeterli artış olmaması klinik tablonun ağırlaşmasından sorumlu bir faktör olarak da göz önünde bulundurulabilir.

Anahtar Kelimeler: Biyobelirteç, hypoxia-inducible factor 1α, SARS-CoV-2

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INTRODUCTION

In December 2019, a new coronavirus, previously called 2019-nCoV, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China. SARS-CoV-2 caused a respiratory disease called Coronavirus 2019 (COVID-19), which was officially named by the World Health Organization (WHO), on February 11, 2020. Interpersonal transmission of coronaviruses mainly occurs through direct and indirect contact with saliva droplets or surfaces. COVID-19 caused serious diseases and deaths in China and other countries around the world.[1-5] Together with severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), COVID-19 appears to cause a serious clinical features in humans, from mild fatigue to death due to sepsis or acute respiratory distress syndrome. The prognosis is worse in accompanying elderly patients. As of today, there is no specific therapy for COVID-19.[6-9] The mechanism of the SARS-CoV-2 infection has not yet understood. The key to human transmission is the virus's ability to bind to human cells: Coronaviruses use the spike proteins to bind to cells and SARS-CoV-2 uses the same angiotensin 2 enzyme (ACE2) receptor as the SARS-CoV.[5,7,10] Clinical features related to SARS-CoV-2 infection varies from mild fatigue to death from sepsis and / or ARDS.[6] The lack understanding of mechanism of the SARS-CoV-2 infection and this variety in clinical features are the leading reasons why a specific therapy has not yet been developed. There are not enough studies in the literature about the mechanism of this new viral infection.

HIF-1 is a heterodimeric receptor with a short half-life, that can be found throughout the body and has been shown to respond to hypoxic conditions. HIF-1 helps the metabolism adapt to and recovery from severe hypoxic conditions such as inflammation, sepsis, hypertension, hypervolemic shock, heart or lung diseases, and anemia. In these critical conditions, HIF-1α dimerizes with transcription factor HIF-1β to copy various hypoxia response genes. In early stages of acute lung injury / ARDS, damage to the alveolar membrane, alveolar epithelial cell apoptosis and pulmonary edema can easily lead to hypoxia and activation of HIF-1α.[11-14] Studies have shown that HIF-1α can control inflammation and alleviate acute lung damage by regulating glucose metabolism in alveolar epithelial cells. It has been reported that HIF-la expression increases after lung contusion, and this HIF-la expression stimulates the proliferation and expansion of type II alveolar epithelial cells to alleviate damage after acute lung injury.[12,15-17]

Acute pulmonary injury in COVID-19 has important clinical findings in both diagnosis and follow-up of the disease. The mechanisms that is effective in the development of this damage have not been elucidated yet. However, the formation of hypoxic conditions, inflammation and sepsis during the course of the infection are main factors that lead to multiple organ injuries. As a protective factor for such clinical conditions, increased expression of HIF-1α may play a role in the development of different clinical features. This study was aimed to answer the questions of whether the serum levels of HIF-1α, which is increased by up to 100 times in many tissues including pulmonary tissue in cases of acute lung injury, could be used as an effective parameter in monitoring the severity and prognosis of the disease in COVID-19 patients.

MATERIALS AND METHODS

Study Population

40 patients and 20 healthy control, who admitted to Tokat Gaziosmanpasa University Hospital between 6 April and 1 May 2020 with COVID-19 clinical symptoms, were included in the study. A suspect case for COVID-19 has been identified as someone who meets both of the following criteria: 1) fever with the presence of at least one these two condition; respiratory symptoms such as cough, sore throat or shortness of breath or radiographic evidence of pneumonia 2) history of contact with COVID-19 patient. A confirmed case was defined as a patient with positive results for the real-time reverse transcription polymerase chain reaction (RT-PCR) test for SARS-CoV-2 in the upper respiratory sample (nasopharyngeal and oropharyngeal swab) or in the lower respiratory sample (without sputum). 56 patients who admitted with COVID-19 clinical complaints, 20 of them formed the confirmed case group, another 20 patients formed the suspect case group. None of the patients in the suspect group developed COVID-19 during clinical and laboratory follow-ups. While forming the control group, individuals were chosen according to the criteria of not having any acute infection or chronic diseases, and having an age and gender distributions similar to other groups.

Clinical Classifications

All cases were divided into four groups according to their clinical symptoms such as severity of pneumonia, respiratory failure, shock, and other organ failures. (1) Mild type: mild clinical symptoms without pneumonia findings in imaging; (2) common type: fever, respiratory symptoms and pneumonia findings in imaging; (3) severe type: respiratory distress, respiratory rate ≥ 30 / min; oxygen saturation 93% at rest; PaO2 / FiO2 ≤ 300 mmHg; (4) critical type: respiratory failure requiring mechanical ventilation, shock and other organ failure requiring ICU monitoring and treatment.

Laboratory Assay

Clinical and laboratory data of COVID-19 group were evaluated at three different times: hospital admission day (day 0), one week after admission (week 1), and disease outcome. For the evaluation of the study parameter, Day 0 and Week 1 blood samples were collected, centrifuged and stored at -80°C. Laboratory evaluation of the patients and the control groups included inflammatory markers and disease-specific markers.

Procalcitonin, C-reactive protein (CRP), Ferritine and D-dimer which are known biochemical markers, were used to assess the disease activity. The HIF-1α serum levels were measured using an enzyme-linked immunosorbent assay commercially available kit (Bioassay Technology Laboratory Human Hypoxia-inducible Factor 1 Alpha ELISA Kit). Other laboratory data were obtained from the hospital information system.

Statistical analysis

The SPSS version 18.0 Windows software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis of the obtained data. Whether the variables show normal distribution or not was analyzed by the Test of Homogeneity of Variances. Since the variables did not show normal distribution, nonparametric tests, which were more suitable than statistical tests, were used. Mann–Whitney U test was used for non-homogenously distributed data and Student T test was used for homogeneously distributed data. Mann-Whitney U test was used in comparison of patients with healthy volunteers as well as in binary group comparisons. The categorical variables were compared using the chi-squared test. Kruskal-Wallis H test was used to compare more than two groups. Spearman correlation analysis was used to determine the relationship between numerical variables. Differences of $p<0.05$ were considered to be statistically significant.

Ethics approval

This research study was approved by Republic of Turkey Ministry of Health (KÖKSAL DEVECİ-2020-05-05T00_55_27) and the Tokat Gaziosmanpasa University clinical research ethics committee (15-KAEK-172) and it was planned and conducted in accordance with the provisions of the Helsinki Declaration.

RESULTS

The mean age of COVID-19 positive, COVID-19 suspect and control groups included in the study were 57.8±16.1, 60.7±19.2 and 61.2±11.9, respectively. 8 (40.0%) patients in the COVID-19 positive group, 10 (50.0%) patients in the COVID-19 suspect group and 7 (35.0%) individuals in the control group were over 65 years old. 35.0% of COVID-19 positive group was male, 65.0% was female, 35.0% male of COVID-19 suspect group was male, 65.0% female, 45.0% male of control group, 55.0% female (**Table 1**).

When the clinical symptoms of admission were evaluated, fever was the most common symptom in the COVID-19 positive group with 73.3%. In 20% of COVID-19 suspect group, fever was the symptom on admission ($p<0.05$). No significant difference was found between these two groups in terms of clinical presentations of dyspnea and cough ($p>0.05$). Chest tightness, sputum and fatigue could not be evaluated due to insufficient number of patients. When chronic disease of the patients were evaluated, it was found that these incidences were higher in the COVID-19 suspect group (60%) than the positive group (33.3%) ($p<0.05$) (**Table 1**).

When laboratory data of all three groups were compared, WBC, lymphocyte, monocyte and platelet counts of the COVID-19 positive group were significantly lower than the COVID-19 suspect group and the control group ($p<0.05$). AST values of COVID-19 positive group were significantly higher than AST values of COVID-19 suspect group and control group. COVID-19 positive group CRP, procalcitonin, ferritin and D-Dimer values were significantl"y higher than the values of COVID-19 suspect group and control group ($p<0.05$). Also, procalcitonin and ferritin values of COVID-19 suspect group were significantly higher than the control group ($p<0.05$) (**Table 1**).

The mean (range) of serum HIF-1α values of the COVID-19 positive, suspect and control groups were 2.27 (1.68-7.97), 2.69 (0.92-11.44) and 1.85 (0.58-2.73), respectively. No significant difference was found between serum HIF-1α values of COVID-19 positive group and COVID-19 suspect group ($p>0.05$). A significant difference was found between serum HIF-1α values of COVID-19 positive group, COVID-19 suspect group and control group ($p <0.05$) (**Table 1**).

In **Table 2**, laboratory results of 20 COVID-19 positive patients on admission (Day 0) and Week 1 after hospitalization were compared. The mean HIF-1α values (3.04±1.75) of Day 0 were significantly higher than the mean HIF-1α values (2.73±1.84) of Week 1 ($p<0.05$). Monocyte count, platelet count and ferritin values of COVID-19 positive patients in the first week after hospitalization were significantly higher than on admission values ($p<0.05$).

Table 3 shows the rates of disease severity of COVID-19 positive and COVID-19 suspect group on the day of admission (Day 0), on the first week after hospitalization and on the day of discharge (end of the disease). For COVID-19 positive group, the rate for mild severity was 15.0% on Day 0, it reached 50.0% in the 1st week and it was 85.0% at the end of the disease. The rate of cases with moderate severity on admission was 70.0%, which dropped to 35.0% in the first week and was 0.0% at the end of the disease. None of the cases were severe on admission to hospital and only 10.0% of cases were severe in the first week. 3 patients (15.0%) disease severity was critical on admission, their status did not change in the first week of the hospitalization and the disease resulted in death for 3 patients (15.0%). In COVID-19 suspect group, disease severity distributions were 12 cases (60.0%) of mild, 3 cases of moderate (15.0%), 5 cases of severe (25.0%) and no case of critical (0.0%).

Serum HIF-1α values of the COVID-19 positive and COVID-19 suspect groups were compared according to the disease severity. The 1st week serum HIF-1α levels of the COVID-19 positive critical/death cases (1.54 (1.14-1.94) was found to be significantly lower than serum levels of day 0 and week 1 of COVID-19 positive mild cases.[4.07 (2.14-6.01) and 3.43 (2.23-4.63), respectively] ($p<0.05$). For COVID-19 suspect group, no significant difference was found between serum HIF-1α values of the mild and severe cases ($p>0.05$) (**Table 4**).

Table 1. Comparison of demographic, clinical and laboratory data of study groups

	COVID-19 positive (n=20)	COVID-19 suspect (n=20)	Control (n=20)
Age (year)	57.8±16.1	60.7±19.2	61.2±11.9*
Gender (M/F)	7/13	7/13	9/11**
Clinical symptoms			
Fever	11 (73.3 %)[b]	3 (20.0 %)**	-
Dyspnea	5 (33.3 %)	7 (46.7 %)**	-
Coughing	11 (73.3 %)	9 (60.0 %)**	-
Chest tightness	0 (0.0 %)	3 (20.0 %)**	-
Sputum	0 (0.0 %)	4 (26.7 %)**	-
Fatigue	4 (26.7 %)	0 (0.0 %)**	-
Chronic Disease	5 (33.3 %)[b]	9 (60.0 %)**	-
Laboratory Tests			
WBC (×10³, cell/mL)	5.07 (3.15-6.50)[a,d]	9.22 (5.36-37.26)[d]	6.60 (3.58-10.09) ***
Lymphocyte (×10³, cell/mL)	1.13±0.53[b,d]	2.06±0.94	2.0±1.04 *
Monocyte (×10³, cell/mL)	0.30 (0.20-0.58)[b,d]	0.51 (0.19-1.71)	0.48 (0.20-1.40) ***
Platelet (×10³, cell/mL)	153.1±55.2[b,c]	233.8±82.1	242.9±63.0 *
AST (U/L)	37.6 (13.5-389.0) [b,d]	21.4 (13.0-43.4)	19.5 (10.0-179.0) ***
ALT (U/L)	24.0 (4.8-198.0)	23.0 (6.9-42.9)	15.0 (8.0-174.0) ***
Total Bilirubin (mg/dL)	0.60 (0.18-0.51)	0.38 (0.20-1.25)	0.50 (0.14-1.04) ***
Creatinine (mg/dL)	0.91±0.19	0.93±0.11	0.87±0.21 *
Na (mmol/L)	140.5±4.9	141.2±4.0	140.2±2.4 *
Cl (mmol/L)	103.7±5.3	103.8±3.6	104.2±2.9
CRP (mg/L)	43.15 (0.06-163.44)[a,c]	3.14 (0.22-159.97)	3.50 (0.54-67.0) ***
Procalcitonin (ng/mL)	0.087 (0.046-7.19)[b,c]	0.061 (0.034-0.179)[c]	<0.020*
Ferritin (ng/mL)	414.8 (96.35-1257.0)[b,c]	72.41 (9.93-227.0)[c]	38.10 (9.72-182.24) ***
D-dimer (mg/L)	0.34 (0.07-8.42)[b,c]	0.22 (0.05-0.97)	0.12 (0.06-0.14) ***
PT (sec.)	16.7±4.5	11.7±7.0	11.9±1.6 *
APTT (sec.)	28.7±5.2	28.6±5.5	32.4±3.6 *
Fibrinogen (mg/dL)	347.5 (113.0-620.0)	274.5 (241.0-308.0)	298.2 (185.0-380.4) ***
HIF-1α (ng/ml)	2.27(1.68-7.97)[c]	2.69 (0.92-11.44)[c]	1.85 (0.58-2.73) ***

* Student T test, ** Chi-squared test, *** Mann-Whitney U test, [a]P <0.001 significant difference from COVID-19 suspect group, [b]P <0.05 significant difference from COVID-19 suspect group, [c]P <0.001 significant difference from control group, [d]P <0.05 significant difference from control group

Table 2. Comparison of HIV-1 α and laboratory results of COVID-19 patients on admission and at the first week of hospitalization

	COVID-19 positive group (Day 0) (n=20)	COVID-19 positive group (Week 1) (n=20)	P value
HIF-1α (ng/ml)	3.04±1.75	2.73±1.84	<0.05*
WBC (×10³, cell/mL)	5.06±1.04	6.16±2.76	>0.05*
Lympochyte (×10³, cell/mL)	1.17±0.56	1.28±0.75	>0.05*
Monocyte (×10³, cell/mL)	0.34± 0.11	0.42±0.17	<0.05*
Platelet (×10³, cell/mL)	150.6±47.5	242.1±76.9	<0.05*
AST (U/L)	33.7 (13.5-119.4)	26.7 (14.4-148.2)	>0.05**
ALT (U/L)	21.9 (4.8-85.9)	35.25 (5.6-210.6)	>0.05**
Total Bilirubin (mg/dL)	0.59±0.39	0.63±0.36	>0.05*
Creatinine (mg/dL)	0.88±0.19	0.96±0.55	>0.05*
Na (mmol/L)	142.2±4.8	144.7±4.4	>0.05*
Cl (mmol/L)	103.9±5.5	106.4±5.7	>0.05*
CRP (mg/L)	30.53 (10.06-163.44)	24.83 (3.35-364.78)	>0.05**
Procalcitonin (ng/mL)	0.086 (0.055-0.544)	0.114 (0.045-17.43)	>0.05**
Ferritin (ng/mL)	391.3 (96.35-1063.0)	508.5 (107.9-1551.0)	<0.05**
D-dimer (mg/L)	0.52 (0.07-8.42)	0.66 (0.15-5.36)	>0.05**
PT (sec.)	17.05±4.9	16.37±2.7	>0.05*
APTT (sec.)	27.41±3.5	25.90±3.8	>0.05*
Fibrinogen (mg/dL)	324.1±96.4	379.2±107.6	<0.05*

* Student T test, ** Mann-Whitney U test

Table 3. Clinical classification of COVID-19 suspect group and COVID-19 positive group

	COVID-19 positive group (n=20)			COVID-19 suspect group (n=20)
	On Admission (Day 0)	In First Week (Week 1)	Hospital Discharge	On Admission (Day 0)
Mild Type	3 (15.0%)	10 (50.0%)	17 (85.0%)	12 (60.0%)
Common Type	12 (60.0%)	3 (15.0%)	0 (0.0%)	3 (15.0%)
Severe Type	2 (10.0%)	4 (20.0%)	0 (0.0%)	5 (25.0%)
Critical Type	3 (15.0%)	3 (15.0%)	3 (15.0%)	0 (0.0%)

Table 4. Comparison of serum HIF-1α levels according to the clinical classification of patient groups

	COVID-19 positive group (n=20)		COVID-19 suspect group (n=20)
Serum HIF-1α levels	On Admission (Day 0)	In First Week (Week 1)	On Admission (Day 0)
Mild Type	4.07 (2.14-6.01)	3.43 (2.23-4.63)	2.81 (0.92-11.44)
Common Type	2.28 (1.68 -7.97)	2.13 (1.20-8.22)	-
Severe Type	2.86 (2.06-4.04)	2.87 (2.05-3.69)	2.86 (2.06-4.04)
Critical Type	2.01 (1.94-2.09)*	1.54 (1.14-1.94)*	-

Mann-Whitney U test, *Significant difference from mild cases of COVID-19 positive group (p <0.05)

Table 5. Correlation analysis of HIF-1α results of patient groups

	COVID-19 positive group				COVID-19 suspect group		Control group	
	On Admission (Day 0) HIF-1α		In First Week (Week 1) HIF-1α		On Admission (Day 0) HIF-1α		HIF-1α	
	r	p	r	p	r	p	r	p
Age	-0.738	<0.001*	-0.579	0.024	0.232	0.405	-705	0.003*
Platelet count	0.594	0.042*	0.392	0.208	-0.271	0.328	262	346

Spearman corerelation analysis, * p<0.05

When the results of the correlation analysis of serum HIF-1α values in the study groups were evaluated, there was a strong negative correlation between the day 0 HIF-1α values and the patient age for COVID-19 positive group, whereas a weak positive correlation was found between the HIF-1α values and platelet count (r; - 0.738, p <0.001 and r; 0.594, p: 0.042, respectively). There was a weak negative correlation between HIF-1α values and the age of the patient only at week 1 (r; -0.579, p: 0.024). In COVID-19 suspected group, no relation was found between serum HIF-1α values and any of the study parameters. In the control group, there was a moderate negative correlation between HIF-1α values and the age (r; -0.705, p: 0.003). (**Table 5**).

DISCUSSION

In order to fight against COVID-19 pandemic, clinical and laboratory determinants of progression to severe and fatal forms need to be urgently identified. At the same time, many studies are needed to explain the mechanisms of the disease and to develop new treatment strategies. In this study, it was found that the serum HIF-1α values in COVID-19 confirmed patient group were higher than serum HIF-1α values of healthy control group. However, no significant difference was found for the COVID-19 suspect group.

On the day of hospitalization, serum HIF-1α values were higher than the HIF-1α values after hospitalization, whereas Monocyte, platelet and ferritin values were lower. Serum HIF-1α values of the critical cases subgroup of the COVID-19 confirmed patient group were lower than the mild cases subgroup's Day 0 and Week 1 values. Within COVID-19 group, HIF-1α values were strongly negative correlated with patient age, whereas platelet numbers were weakly positive correlated.

Clinically, patients with SARS had a triphasic disease pattern, such as fever, nonproductive cough, sore throat, and muscle pain.[18] In this study, COVID-19 patients had fever (73.3%), cough (73.3%), dyspnea (33.3%), and weakness (26.4%). The new coronavirus (COVID-19) pneumonia outbreaked at the end of 2019 is highly contagious, with a raw mortality rate of about 2.3%.[18] Approximately 80.9% of patients are mildly to moderately ill and have a better prognosis. However, the mortality rate increased significantly for patients who developed severe or critical levels and the raw mortality rate reached 49% in critical patients.[19] In this study, the cases 65% of the cases were mild and moderate, and 35% of them were severe or critical. The mortality rate for severe or critical patients was 42.8%. The main clinical signs of COVID-19indicate fever (90% or more), cough (about 75%) and dyspnea (up to 50%). A small but important subset has gastrointestinal symptoms..[7, 20-23]

The mechanism of the SARS-CoV-2 infection is not yet known. "Cytokine storm" or "cytokine cascade" are among the default mechanisms for organ damage. Various recent studies have linked some of biomarkers to a severe disease progression.[24] In this study, HIF-1α was evaluated both as its role in disease mechanism and as a biomarker. COVID-19 is a viral disease characterized by normal or low white blood cell count and decreased lymphocyte count. Among the hematological parameters, lymphopenia is clearly associated with disease severity. Patients who died from COVID-19have significantly lower lymphocyte counts than survivors.[25.26] In terms of laboratory tests, Zhang et al. found a decrease in WBC count in 38.66% of patients and a decrease in lymphocyte count in half of patients (48.45%). At the same time, CRP, ferritin, procalcitonin and D-dimer levels increased in a significant number of patients in relation to the severity of the disease.[25] It has been reported that especially in some patients with multiple organ failure, alanine aminotransferase, aspartate aminotransferase (liver failure), creatine kinase and lactate dehydrogenase, troponin (myocarditis), urea and creatinine (kidney failure) levels and cagulation parameters could increase. In severe cases, elevation in these parameters may be seen initially before multiorgan failure develops.[25,26]

In our study, WBC, lymphocyte, monocyte and platelet counts were lower in the COVID-19 positive group than in the COVID-19 suspect group and the control group. WBC values of the COVID-19 suspect group increased compared to the control group. AST, CRP, ferritin, procalcitonin and D-dimer levels, which were among other parameters, increased in the COVID-19 group compared to the suspect group and the control group. Suspect group procalcitonin and ferritin values also increased compared to the control group. These results support the studies that shows these parameters can be used to separate COVID-19 patients from the suspect group and the healthy group. However, when we evaluated the changes of these parameters after the first week of hospitalization of COVID-19 patients, a significant change was observed only in the monocyte, platalet counts and procalcitonin values. These findings led us to the conclusion that monocyte count, platalet count and procalcitonin levels could be more useful as follow-up tests for COVID-19 patients.

HIF-lα has been shown to regulate the expression of more than 100 downstream genes during acute hypoxia, protecting it from hypoxic stress in many ways, most of which affect the progression of inflammation.[12,17] In early stages of acute lung injury / ARDS, damage to the alveolar membrane, alveolar epithelial cell apoptosis and pulmonary edema can easily lead to hypoxia and activation of HIF-1α. Studies have shown that HIF-1α can control inflammation and alleviate acute lung damage by regulating glucose metabolism in alveolar epithelial cells.[12, 13] It has been reported that HIF-la expression increases after lung contusion, and this HIF-1α expression stimulates the proliferation and expansion of type II alveolar epithelial cells to alleviate damage after acute lung injury.[14, 27, 29] HIF-1 has also been found to be active in various epithelial tissues during trauma and infection. In their study, Sherman et al. investigated the responses of alveolar epithelial cells to lung contusions and they found an increase in expression of HIF-1α in the lungs in 48 hours in the lung and in 24 and 48 hours in the liver.[15] Matsuishi et al. in their study which they aimed to reconstruct acute lung injury by administering lipopolysaccharide in a rat model to examine early sepsis-related recovery, improvement and complications, reported that there was a significant levels of expression of HIF-1α mRNA in the untreated group compared to the group that treated with Lindiolol, a beta receptor blocker.[17]

Lung contusions are a risk factor and one of the causes of acute respiratory distress syndrome, in which fluid collects in the alveoli.[13] During acute respiratory distress syndrome, epithelial cells, especially alveolar type (AT) I cells, disappear, resulting in increased permeability. The fluid collection prevents gas exchange and can lead to both local and systemic hypoxia. Hypoxia is the driving force of inflammation. HIF-la is the key mediator of the inflammatory response following lung contusions, but can also be induced by inflammation that begins with hypoxia or other physiological disruptions. [30] ATII cells proliferate and get distributed over the decayed basement membrane to reseal the barrier. Repair of the alveolar epithelium is critical for clinical recovery. It is thought that the hypoxia-related factor (HIF) la supports the proliferation and spread of ATII cells during post-lung injury repair.[16]

In this study, an increase in serum HIF-1α levels was found in the COVID-19 positive group compared to the healthy control group. However, since this increase was also in the COVID-19 suspect group, there was no significant difference between the COVID-19 positive group and the COVID-19 suspect group. When we re-evaluated the samples of COVID-19 group after 1 week, a significant decrease was found in the values. When we compare HIF-1α levels according to the severity of these patients, we found that critical patients' HIF-1α levels were significantly lower than patients having mild disease course. At the same time, serum HIF-1α levels of COVID-19 patients were strongly negative correlated with patient age, whereas platelet counts were weakly correlated. These findings lead us to the conclusion that that there is an increase in HIF-1α levels in COVID-19 disease, but this increase is not a specific for COVID-19. Instead, the lack of adequate increase in HIF-1α levels in patients with critical condition may be determining factor in the course of the disease because, 3 critical COVID-19 patients, whose HIF-1α levels were low, didn't survive. An increase of HIF-1α in mild and moderate COVID-19 patients may prevent the progression of pulmonary damage, through stimulating the proliferation of type II alveolar cells. On the other hand, low HIF-1α levels in severe and critical patients can lead to the propagation of alveolar damage and multiple organ failure. The strong negative correlation between age and HIF-1α should be considered as another factor that may determine the severity of COVID-19 in elderly patients.

CONCLUSIONS

COVID-19 is a dangerous and severe disease, the mechanism of the emergence and progression is currently unclear, and therefore detailed study is required. In this study, monocyte, platelet and ferritin tests were illustrated that they are important biomarkers in disease follow-up. Serum levels of HIF-1α, which are thought to prevent alveolar damage, were increased in patients with COVID-19. However, low levels of serum HIF-1α should be considered as a factor responsible for alveolar damage in critically ill patients.

Limitations of study

The limitations of this study is that this study included a small number of patients from a single center. As more data are gathered from prospective studies with longer follow-ups, these findings should be reassessed continuously in upcoming months.

ETHICAL DECLARATIONS

Ethics Committee Approval: This research study was approved by Republic of Turkey Ministry of Health (KÖKSAL DEVECİ-2020-05-05T00_55_27) and the Tokat Gaziosmanpasa University clinical research ethics committee (15-KAEK-172) and it was planned and conducted in accordance with the provisions of the Helsinki Declaration.

Informed Consent: Written consent was obtained from all patients who participated in the study and their relatives.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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