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Original research article

Evaluation of IGFBP5 expression and plasma osteopontin level in COVID-19 patients

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ABSTRACT
Purpose: The aim of this study is to investigate insulin-like growth factor binding protein 5 (IGFBP5) expression in coronavirus disease 2019 (COVID-19) patients and its relationships with COVID-19 laboratory findings and plasma osteopontin (OPN) levels.

Materials and methods: We enrolled 60 patients with COVID-19 and 30 healthy individuals in this study. mRNA expression of IGFBP5 was measured by RT-PCR. Plasma OPN levels were measured via the ELISA method.

Results: Plasma OPN levels were higher and IGFBP5 expression levels were lower in COVID-19 patients than in the healthy individuals (p = 0.0057 and p = 0.0142, respectively). Critically ill patients had higher OPN and lower IGFBP5 than non-critically ill patients. Patients with affected lungs demonstrated increased OPN and decreased IGFBP5 (p = 0.00032 and p = 0.044, respectively). Receiver operating characteristic (ROC) analysis indicated that IGFBP5 expression and OPN levels can be used discriminate non-critically from critically ill patients (p = 0.049; p = 0.0016, respectively).

Conclusion: This study demonstrated that patients with a poor prognosis had increased OPN and decreased IGFBP5. High values of OPN and low values of IGFBP5 may be considered as signs of disease severity. Tissue-specific IGFBP5 expression may contribute to understanding the role of IGFBP5 in the lungs in COVID-19 cases.

1. Introduction

Coronavirus disease 2019 (COVID-19) causes the death of patients for many known and unknown reasons [1]. The hospitalization rate varies according to published studies. Disease symptoms, hospitalization rates, clinical severity, drugs used, and mortality rates have been investigated since the beginning of the pandemic. More recently, researchers have focused on exploring the molecular and genetic changes caused by the disease. The severity of the disease can range from mild cold symptoms to respiratory failure requiring mechanical ventilation and even ending in death [2]. The disease can become more severe with pre-existing conditions such as obesity, smoking, advanced age, and various chronic diseases [3]. Specific treatment for COVID-19 has not yet been found, and many patients continue to suffer the effects of the disease even after treatment has ended [4]. Many organs, including the lungs, liver, and kidneys, may be affected by COVID-19 infection. Pulmonary fibrosis was shown in patients with COVID-19 even after they were discharged from the hospital [4].

The insulin-like growth factor (IGF) axis has several members, including IGFs, receptors, and IGF binding proteins (IGFBPs) [5]. The IGF-1 pathway may have a regulator role in the immune response through cytokines, immune cells, and bone [6]. IGFBPs regulate IGF activities by binding or not binding to IGFs [7]. As a conserved member of the IGF axis, IGFBP5 is expressed in many tissues, including bone, lung, ovary, and mammary tissues [5,8]. IGFBP5 promotes or inhibits cell proliferation, survival, and migration in IGF-dependent and IGF-independent manners in many different types of tissues and cells [9]. IGFBP5 was upregulated in the lungs of patients with idiopathic pulmonary fibrosis [10]. IGFBP5 stimulated extracellular matrix (ECM) components and profibrotic genes in vitro [10,11]. Furthermore, it has a positive feedback loop that induces its...
expression [11]. IGFBP5 is also involved in the immune response and it induces migration of peripheral blood mononuclear cells, including monocytes, T cells, and natural killer cells [12].

Osteopontin (OPN) is a glycoprotein that plays a role in lymphocyte differentiation and inhibits apoptosis in inflammatory cells [13]. It was found to be expressed in many cells and organs, including leukocytes, bone cells, breast epithelial cells, the blood and lungs [13,14]. OPN has essential roles in monocyte activation, cytokine release, cell migration, and differentiation in the inflammation process [15]. OPN levels are increased in patients with systemic sepsis [16]. In severe cases of COVID-19, plasma OPN levels were higher than those seen in non-severe cases [17]. OPN regulates pulmonary fibrosis as a fibrotic cytokine [18].

IGFBP5 and OPN are linked to bronchopneumonia as a part of the process of COVID-19 infection. Uncov- brosis is a part of the process of COVID-19 infection. Pulmonary fibrosis as a bronchopneumonic cytokine [18].

2. Material and methods

2.1. Study design

This study included 90 individuals divided into three groups. Thirty individuals were healthy controls, while 60 (30 non-critically ill patients and 30 critically ill patients) were COVID-19 patients. Thirty of those patients had only flu-like symptoms, but 30 patients were critically ill and required intensive care unit admission due to COVID-19 infection. Blood samples were collected at the Department of Infectious and Clinical Microbiology and Department of Chest Diseases of Regional Training and Research Hospital (Erzurum, Turkey). Blood samples were taken from patients after they presented to hospital and were diagnosed with COVID-19 infection. Patient demographic data and laboratory findings were obtained from hospital records.

2.2. Plasma samples and ELISA

Blood samples were taken into tubes containing ethylenediaminetetraacetic acid. The plasma of the participants’ blood was centrifuged at 4 °C and 4000 rpm for 10 min. Plasma samples were stored at −80 °C until measurements were performed. Plasma OPN level was measured by enzyme-linked immunosorbent assay (ELISA; Elabscience, Texas, USA) according to the manufacturer’s instructions with the Epoch Microplate Spectrophotometer System and Take3 Plate (BioTek, Santa Clara, CA, USA).

2.3. RNA extraction and cDNA synthesis

According to the manufacturer’s instructions, total RNA was extracted from 100 μL of peripheral blood samples with the EcoPURE Total RNA Kit (EcoTech Biotechnology, Erzurum, Turkey). RNA was measured spectrophotometrically with the Epoch Microplate Spectrophotometer System and Take3 Plate (BioTek, Santa Clara, CA, USA). RNA (10 μg) was used for cDNA synthesis with the iScript cDNA synthesis kit (Bio-Rad, Feldkirchen, Germany). The total volume of PCR mix was 20 μL, which included 8 μL of RNA, 4 μL of iScript reaction mix, 1 μL of iScript Reverse Transcriptase, and 7 μL of nuclease-free water. The cDNA synthesis program ran for 5 min at 25 °C (priming), 20 min at 46 °C (reverse transcription), and 1 min at 95 °C (RT inactivation).

2.4. Gene expression analysis

Real-time PCR (RT-PCR) was performed with iTaq Universal SYBR Green Supermix in the Applied Biosystems StepOnePlus™ Real-Time

| Table 1 |
| --- |
| Laboratory findings of patients. Data presented as mean ± standard deviation (SD). |
| | Non-critically ill patients (mean ± SD) | Critically ill patients (mean ± SD) | p value |
| Age(years) | 45.86 ± 4.307 | 71.92 ± 2.211 | <0.0001 |
| White blood cell count (x103/L; normal range; 4.49–12.68) | 7.110 ± 0.3316 | 8.933 ± 0.7262 | 0.0375 |
| Neutrophils* (x 109/L; normal range; 2.04–7.54) | 4.198 ± 1.343 | 6.691 ± 3.619 | 0.0331 |
| Lymphocytes (x 109/L; normal range; 1.21–3.77) | 2.092 ± 0.1473 | 1.102 ± 0.1337 | <0.0001 |
| Platelets* (x 109/L; normal range; 152–383) | 240.9 ± 53.19 | 202 ± 96.28 | 0.0188 |
| Hemoglobin (g/dL; normal range; 12.2–15.9) | 14.55 ± 0.4167 | 13.09 ± 0.3935 | 0.0147 |
| Hematocrit (%) (% normal range: 36.4–47.2) | 44.55 ± 1.201 | 41.75 ± 1.138 | 0.098 |
| Albumin (g/L; normal range 32–48) | 45.62 ± 0.788 | 37.2 ± 1.356 | <0.0001 |
| Alanine aminotransferase* (U/L; normal range 7–40) | 32.7 ± 20.28 | 60.88 ± 85.12 | 0.0692 |
| Aspartate aminotransferase (U/L; normal range 13–40) | 33.3 ± 20.68 | 66.52 ± 94.65 | 0.0182 |
| Total bilirubin* (mg/dL; normal range 0.2–1.1) | 0.5190 ± 0.1505 | 1.078 ± 1.205 | 0.0469 |
| Lactate dehydrogenase* (U/L; normal range 120–246) | 224.2 ± 126 | 483.8 ± 184.5 | <0.0001 |
| Procalcitonin* (ng/mL; normal range 0–0.5) | 0.6141 ± 0.5359 | 2.975 ± 7.202 | 0.0102 |
| C-reactive protein* (mg/L; normal range 0–0.5) | 13.67 ± 25.44 | 4902 ± 24143 | 0.0004 |
| D-dimer* (μg/mL; normal range; 0–500) | 482.4 ± 431.4 | 4136 ± 8029 | 0.0002 |
| Creatinine* (mg/dL; normal range; 0.55–1.02) | 0.7205 ± 0.2517 | 1.153 ± 0.5654 | 0.0004 |
| Fibrinogen (mg/dL; normal range; 200–400) | 406.7 ± 26.87 | 527.9 ± 46.35 | 0.0348 |
| Ferritin* (ng/mL normal range; 10–291) | 252.6 ± 414.4 | 1419 ± 4454 | 0.0112 |

Student t-test and Mann Whitney U test were used to compare the groups. Bolded values indicate p < 0.05 which was considered statistically significant.

PCR System (Foster City, CA, USA) according to the manufacturer’s guidelines. The PCR mix included 10 μL of supermix, 2 μL of primers (forward and reverse), 4 μL of cDNA, and 4 μL nuclease-free water. β-Actin was used as the housekeeping gene. The program for RT-PCR was run at 95 °C for 30 s (polymerase activation and DNA denaturation), at 95 °C for 15 s (denaturation), and at 60 °C for 60 s (annealing/extension and plate reading) for 40 cycles. All reactions were run in triplicate. IGFBP5 gene expression was determined by the 2−ΔΔCT method [19]. Primer sequences were as follows:

IGFBP5
Forward Primer: 5’-AAGAAGCTGACCACTCAGCA-3’.
Reverse Primer: 5’-GATCTTTCGGCTACATCAAT-3’.

β-Actin
Forward Primer: 5’-CATGTACGTTCGTACCCAGCCG-3’. Reverse Primer: 5’-CTCCTTAATGTCACCGCAG-3’.

2.5. Statistical analysis

GraphPad Prism Version 5 (https://www.graphpad.com/) was used for data analysis. Kolmogorov-Smirnov/Shapiro-Wilk tests were applied to test the distribution of the data. Results were given as mean ± standard deviation (SD). Mann-Whitney U test and Kruskal-Wallis test were used to analyze the mRNA expression of IGFBP5 and plasma OPN levels between two groups and more than two groups, respectively. Differences between critically ill patients and non-critically ill patients were analyzed with the Student t-test as a parametric test and the Mann-
Whitney U test as a non-parametric test. Differences in IGFBP5 and OPN levels according to the laboratory findings were evaluated by Student t-test and Mann Whitney U test. Spearman’s r test was applied to calculate the correlation of IGFBP5 expression and OPN level. Receiver operating characteristic (ROC) analysis was performed to analyze the ability of OPN and IGFBP5 to distinguish healthy controls from patients and non-critically ill patients from critically ill. Values of p < 0.05 were considered statistically significant.

2.6. Ethical Issues

This study was approved by the Local Ethics Committee (Date: 04.03.2021, Decision No: 4).

Written informed consent was obtained from all participants.

This study which involves human participants is in compliance with the 1964 Helsinki declaration and its later amendments.

3. Results

3.1. Characteristics and laboratory findings of participants

In this study, 60 patients and 30 healthy individuals were enrolled. The number of female patients was 23 (38%), while the number of male patients was 37 (62%). The mean ages of patients and healthy individuals were 60 and 54 years, respectively. Five of the 60 patients (8%) died. The lungs of 29 patients (48%) were affected by COVID-19 infection. The number of female patients was 23 (38%), while the number of male patients was 37 (62%)

OPN levels were significantly higher in patients than in healthy controls (p = 0.0057) (Fig. 1A). IGFBP5 expression, on the other hand, was significantly lower in patients (p = 0.0019) (Fig. 1B). Participants were evaluated in three groups i.e. control, non-critically ill, and critically ill individuals and significant differences were observed for OPN and IGFBP5 (p = 0.0019 and p = 0.0342, respectively). Critically ill patients showed the highest OPN levels among all participants (Fig. 1C), while IGFBP5 expression was the lowest in the critically ill patients (Fig. 1D).

3.2. OPN level and IGFBP5 expression in patients

Levels of plasma OPN and IGFBP5 expression are presented in Fig. 1. OPN levels were significantly higher in patients than in healthy controls (p = 0.0057) (Fig. 1A). IGFBP5 expression, on the other hand, was significantly lower in patients (p = 0.0019) (Fig. 1B). Participants were evaluated in three groups i.e. control, non-critically ill, and critically ill individuals and significant differences were observed for OPN and IGFBP5 (p = 0.0019 and p = 0.0342, respectively). Critically ill patients showed the highest OPN levels among all participants (Fig. 1C), while IGFBP5 expression was the lowest in the critically ill patients (Fig. 1D).

3.3. Relation of OPN and IGFBP5 expression to laboratory findings

The relationships of OPN and IGFBP5 with laboratory findings are summarized in Table 2. Patients over 50 years of age and patients with affected lungs had increased OPN levels. Patients with high WBC, NEU, AST, LDH, procalcitonin, CRP, D-dimer, creatinine, fibrinogen, and ferritin levels had increased plasma OPN levels and these findings were statistically significant. Patients with low LYM and HG values also had statistically significantly increased OPN levels. Patients who had affected lungs and patients with low LYM values had statistically significantly diminished IGFBP5 expression. Patients with high levels of bilirubin, LDH, D-dimer, and fibrinogen had statistically significantly decreased IGFBP5 expression.

3.4. Correlation of OPN level and IGFBP5 expression

The correlation of OPN and IGFBP5 was evaluated in the 60 COVID-19 patients (non-critically ill and critically ill) (Fig. 2). There was a significant negative correlation between plasma OPN level and IGFBP5 expression in these patients (p = 0.0046, r = 0.4955).
Table 2
Osteopontin level and IGFBP5 expression according to demographic and clinical parameters.

| Parameters                              | Osteopontin (ng/ml) (mean ± SD) | p value | IGFBP5(2.4.4.5) (mean ± SD) | p value |
|-----------------------------------------|----------------------------------|---------|-----------------------------|---------|
| **Age (years)**                         |                                  |         |                             |         |
| <50                                     | 14.3 ± 12.3                      | 0.0196  | 2.7 ± 2.5                   | 0.0652  |
| ≥50                                     | 125.9 ± 10.4                     |         | 0.1 ± 0.99                 |         |
| **Sex**                                 |                                  |         |                             |         |
| Female                                  | 21.4 ± 3.3                       | 0.9479a | 2.3 ± 2.9                   | 0.4903  |
| Male                                    | 21.8 ± 3.48                      |         | 1.37 ± 1.43                |         |
| **Affected Lung**                       |                                  |         |                             |         |
| Normal                                  | 30.5 ± 5.95                      | 0.0032  | 1.055 ± 1.057              | 0.044   |
| Unaffected Lung                         | 14.4 ± 11.6                      |         | 2.52 ± 1.46                |         |
| **White blood cell count** (x10^9/L; normal range 4.49-12.68) | | | | |
| Normal                                  | 18.9 ± 11.2                      | 0.0223  | 2.305 ± 2.48               | 0.077   |
| High                                    | 33.9 ± 5.9                       |         | 0.61 ± 0.54                |         |
| **Neutrophils** (x 10^9/L; normal range 2.04-7.54) | | | | |
| Normal                                  | 17 ± 11                           | 0.01    | 1.47 ± 1.72                | 0.5628  |
| High                                    | 30.9 ± 6.8                        |         | 1.24 ± 1.03                |         |
| **Lymphocytes** (x 10^9/L; normal range 1.21-3.77) | | | | |
| Low                                     | 31 ± 7                            | 0.0242  | 0.95 ± 1                   | 0.0345  |
| Normal                                  | 19 ± 12                           |         | 2.14 ± 2.15                |         |
| **Platelets** (x 10^9/L; normal range152-383) | | | | |
| Low                                     | 31 ± 7                            | 0.1325  | 0.96 ± 1                   | 0.3528  |
| Normal                                  | 20 ± 12                           |         | 1.97 ± 2.5                 |         |
| **Hemoglobin** (g/dL; normal range 12.2-15.9) | | | | |
| Low                                     | 29.1 ± 10                         | 0.0355  | 1.7 ± 1.1                  | 0.1081  |
| Normal                                  | 16 ± 11                           |         | 1.08 ± 1.1                 |         |
| **Hematocrit** (%) (normal range 36.4-47.2) | | | | |
| Normal                                  | 18.76 ± 12.05                     | 0.9728  | 2.03 ± 2.801               | 0.8601  |
| High                                    | 21.68 ± 12.89                     |         | 1.514 ± 1.781              |         |
| **Albumin** (g/L; normal range 32-48)   |                                  |         |                             |         |
| Normal                                  | 22.25 ± 11.82                     | 1.1523  | 1.632 ± 2.373              | 0.1213  |
| High                                    | 15.30 ± 15.29                     |         | 3.065 ± 2.44               |         |
| **Alanine aminotransferase** (U/L; normal range 7-40) | | | | |
| Normal                                  | 18.05 ± 13.13                     | 0.196   | 1.976 ± 2.541              | 0.5025  |
| High                                    | 25.99 ± 9.078                     |         | 1.568 ± 1.923              |         |
| **Aspartate aminotransferase** (U/L; normal range 13-40) | | | | |
| Normal                                  | 15.24 ± 13.34                     | 0.024   | 1.909 ± 2.759              | 0.6452  |
| High                                    | 28.44 ± 7.15                      |         | 1.728 ± 1.762              |         |
| **Total bilirubin** (mg/dL; normal range 0.2-1.1) | | | | |
| Normal                                  | 19.57 ± 12.96                     | 0.2099  | 2.311 ± 2.562              | 0.0415  |
| High                                    | 28.6 ± 6.15                       |         | 0.8188 ± 0.9308            |         |
| **Lactate dehydrogenase** (U/L; normal range 120-246) | | | | |
| Normal                                  | 13.56 ± 10.44                     | 0.0002  | 2.964 ± 3.117              | 0.0385  |
| High                                    | 30.85 ± 5.803                     |         | 0.9949 ± 0.9949            |         |
| **Procalcitonin** (ng/mL; normal range 0-0.5) | | | | |
| Normal                                  | 18.69 ± 10.97                     | 0.0311  | 1.828 ± 1.982              | 0.4315  |
| High                                    | 30.83 ± 6.04                      |         | 1.159 ± 1.225              |         |
| **C-reactive protein** (mg/L; normal range 0-0.5) | | | | |
| Normal                                  | 9.426 ± 10.81                     | 0.0153  | 1.48 ± 1.369               | 0.7475  |
| High                                    | 27.43 ± 7.607                     |         | 1.884 ± 2.451              |         |
| **D-dimer** (µg/ml; normal range 0-500) | | | | |
| Normal                                  | 17.94 ± 11.93                     | 0.0276  | 2.604 ± 2.572              | 0.0403  |
| High                                    | 27.56 ± 9.818                     |         | 0.9722 ± 0.9426            |         |
| **Creatinine** (mg/dL; normal range 0.55-1.02) | | | | |
| Normal                                  | 19.4 ± 11.09                      | 0.0235  | 2.267 ± 2.831              | 0.4687  |
| High                                    | 29.65 ± 10.9                      |         | 1.278 ± 1.327              |         |
| **Fibrinogen** (mg/dL; normal range 200-400) | | | | |
| Normal                                  | 12.49 ± 12.54                     | 0.0158  | 2.478 ± 2.865              | 0.2939  |
| High                                    | 27.12 ± 9.251                     |         | 1.431 ± 1.854              |         |
| **BUN** (mg/dL; normal range 9-23)      |                                  |         |                             |         |
| Normal                                  | 16.84 ± 11.24                     | 0.0019  | 2.175 ± 2.6821             | 0.4996  |
| High                                    | 32.82 ± 5.637                     |         | 1.266 ± 1.276              |         |
| **Ferritin** (ng/ml; normal range 10-291) | | | | |
| Normal                                  | 17.53 ± 11.83                     | 0.0043  | 1.736 ± 1.805              | 0.1779  |
| High                                    | 31.56 ± 6.1                       |         | 1.069 ± 1.152              |         |

Bolded values indicate p<0.05 which was considered statistically significant.

* Student t-test and Mann Whitney U test were used to compare the groups.

3.5. ROC analysis of OPN and IGFBP5

Area under curve (AUC) values were calculated by ROC analysis for OPN and IGFBP5 in non-critically ill patients and critically ill patients (Fig. 3). IGFBP5 was decreased in COVID-19 patients. We investigated whether low IGFBP5 could be a marker of COVID-19 infection. ROC analysis of IGFBP5 indicated an AUC of 0.704 (p = 0.0052) (95%CI 0.5791 to 0.8305) to distinguish patients from healthy controls. Results demonstrated a cut-off value of <1.025 with sensitivity of 0.575 and specificity of 0.769. ROC analysis of IGFBP5 indicated AUC as 0.676 (p = 0.049) (95%CI 0.5717 to 0.8339) for discriminating non-critically patients from critically ill patients. Cut-off value was <0.3769 with
sensitivity of 0.448 and specificity of 0.882. To investigate whether the OPN level can be used to determine the presence and severity of the disease, we calculated the AUC values of the patients’ OPN levels. AUC value was 0.6875 (p = 0.03827) (95%CI 0.531 to 0.844) and cut-off >14.77 ng/ml demonstrated sensitivity of 0.775 and specificity of 0.571 for OPN to discriminate patients from controls. Analysis of non-critically ill and critically ill patients indicated an AUC value of 0.7968 (p = 0.0016) (95%CI 0.645 to 0.949). Cut-off value was determined as >18.75 ng/ml with sensitivity of 1 and specificity of 0.588 for OPN.

4. Discussion

The COVID-19 pandemic remains a global public health problem that may require hospitalization and intensive care treatment. Non-specific laboratory markers are not enough to predict patient prognosis. Due to the variable clinical course of COVID-19, new specific markers are needed in addition to classical clinical and laboratory findings. In this study, we investigated IGFBP5 mRNA expression and OPN plasma levels in COVID-19 patients. The relation of laboratory findings with IGFBP5 and OPN was also explored.

Routine laboratory findings are used to follow patient prognosis. Increased levels of NEU, WBC, D-dimer, CRP, and ferritin and decreased levels of LYM and albumin were previously shown in critically ill COVID-19 patients [17]. These findings may be related to the poor prognosis of such patients [17]. In this study, we found WBC, NEU, ALT, AST, bilirubin, procalcitonin, LDH, CRP, D-dimer, creatinine, fibrinogen, and ferritin values to be higher in critically ill patients than in non-critically ill patients. Lower values of LYM, PLT, HG, HCT, and albumin were also found in critically ill patients.

IGFBP5 functions in the immune response by inducing migration of peripheral immune cells [12]. Lang et al. [20] demonstrated that endotoxin and sepsis decreased IGFBP5 mRNA expression in the gastrocnemius muscle both time- and dose-dependently. Vastrad et al. [21] suggested IGFBP5 as a downregulated gene in COVID-19 infection based on bioinformatics analysis. In line with those studies, we found lower IGFBP5 levels in patients with COVID-19 than in healthy individuals. Furthermore, IGFBP5 expression levels were lower in critically ill patients than in non-critically ill patients. ROC curve of IGFBP5 in this study was calculated to discriminate patients from healthy controls. The AUC

![Fig. 2. Correlation of IGFBP5 and Osteopontin in COVID-19 patients. There was a significant negative association between the IGFBP5 and plasma levels of Osteopontin in COVID-19 patients. Abbreviations: COVID-19 - coronavirus disease 2019; IGFBP5 - insulin-like growth factor binding protein 5.](image)

![Fig. 3. (A) ROC analysis of IGFBP5 between healthy controls and patients and (B) non-critically ill and critically ill patients. (C) ROC analysis of osteopontin between healthy controls and patients and (D) non-critically ill and critically ill patients. Abbreviations: IGFBP5 - insulin-like growth factor binding protein 5; OPN - osteopontin.](image)
value was 0.7048 with sensitivity of 0.575 and specificity of 0.77. ROC curve analysis of non-critically ill patients and critically ill patients’ results demonstrated 0.68 AUC value with 0.44 sensitivity and 0.88 specificity. IGFBP5 tends to decrease in cases of infectious diseases and it has an inverse relationship with increased disease severity in COVID-19 patients. Thus, decreasing IGFBP5 expression level may be a sign of infection and disease progression.

OPN levels were studied in the serum of COVID-19 patients. Varm et al. [17] studied the serum OPN levels of COVID-19 patients and showed higher OPN levels in critically ill patients than in non-critically ill patients. They related those high OPN levels to activated WBC (i.e., NEU, eosinophils, NK cells, LYM, and dendritic cells) [17]. Hayek et al. [22] demonstrated higher levels of OPN in COVID-19 patients than in a control group. They also related elevated levels of OPN to a need of mechanic ventilation. They stated that OPN cannot be used as a biomarker to support the diagnosis of SARS-CoV-2 infection, but it can rather be used to classify patients according to their clinical course [22]. Reisner et al. [23] showed that the OPN levels in children hospitalized with moderate/severe COVID-19 and multisystem inflammatory syndrome in children (MIS-C) were significantly higher than the OPN levels of mild/asymptomatic children. In the present study, we showed increased plasma OPN levels in patients compared to healthy individuals. Critically ill patients had higher OPN levels than non-critically ill patients. ROC analysis results indicated that OPN may be used to discriminate patients from healthy controls, and non-critically ill patients from critically ill patients. OPN may be suggested as a biomarker for COVID-19 infection and may be related to COVID-19 infection and disease severity.

The relationships between OPN and laboratory findings were studied before. Varm et al. [17] found a positive correlation between serum OPN levels and serum inflammatory markers including D-dimer, CRP, WBC, and procalcitonin. Significant correlations of circulation OPN levels and CRP, procalcitonin, ferritin, and D-dimer were shown in another study [22]. In this study, we found increased OPN levels in patients with higher WBC, procalcitonin, CRP, and D-dimer levels. Furthermore, we found increased OPN levels in patients with higher levels of NEU, AST, LDH, creatinine, fibrinogen, and BUN and lower LYM and HG. OPN may be used as a routine laboratory finding in the treatment and follow-up of COVID-19 patients.

It may also be suggested that IGFBP5 plays a role in the pathogenesis of COVID-19 due to its functions in the immune response. In this study, it was observed that IGFBP5 expression was decreased in individuals who had laboratory values that are considered poor for COVID-19 patients. Patients with low LYM and high bilirubin, LDH, D-dimer, and fibrinogen levels had decreased IGFBP5 expression. In addition to those statistically significant findings, it was also observed that IGFBP5 expression was decreased in the event of other abnormal laboratory findings. Thus, IGFBP5 has a link with COVID-19 infection, as evidenced by laboratory findings.

OPN is upregulated in cases of fibrosis. As a fibrotic cytokine, OPN promotes fibroblast migration, proliferation, and adhesion in bleomycin-induced lung fibrosis [18]. Pardo et al. [24] demonstrated that OPN was localized in alveolar epithelial cells in cases of idiopathic lung fibrosis and suggested that OPN is a profibrotic molecule. In the present study, OPN levels were relatively higher in patients whose lungs were affected than in those whose lungs were not affected. In addition to lung fibrosis, increased lung inflammation may also cause such an increase. Nguyen et al. [11] reported the profibrotic activity of IGFBP5 as it stimulates the expression of ECM and profibrotic genes. Pilewski et al. [10] showed increased IGFBP5 mRNA and protein expression in the lungs of patients with idiopathic pulmonary fibrosis and in primary lung fibroblast cultures. However, we found decreased IGFBP5 expression in patients with affected lungs. IGFBP5 expression may change according to tissue type. Local tissues rapidly reflect the alteration of gene expressions, while peripheral blood may not reflect changes in lung tissues.

IGFBP5 stimulates ECM components and profibrotic genes in primary fibroblasts in cases of idiopathic pulmonary fibrosis in vitro [11]. IGFBP5 interacts with ECM proteins including OPN [25]. Kim et al. [26] demonstrated a stimulatory effect of IGFBP5 on OPN expression in MC3T3-E1 cells. However, Durant et al. [27] revealed IGFBP5 overexpression in cases of decreased OPN expression in MC3T3 cells. In COVID-19 patients, IGFBP5 and OPN showed a negative correlation. IGFBP5 expression patterns may change depending on the tissue or cell type as IGFBP5 interacts with different proteins in different cells and/or tissues. In the peripheral blood of COVID-19 patients, IGFBP5 expression decreased and showed a negative correlation with plasma OPN levels.

4.1. Limitations of the study

This study has some limitations. The number of patients was smaller than planned and IGF-1 and other IGFBPs were not investigated. If fibrotic lung tissues were obtained and examined for gene expression, it would be possible to obtain more information about IGFBP5 expression. It is important to take and evaluate blood samples at regular intervals to observe how OPN and IGFBP5 change from the early stages of the disease to the advanced stages of the disease, in order to more clearly reveal the role of these molecules in the course of the disease. In this study, we could not do this as we could only draw blood from patients once.

5. Conclusions

In this study, IGFBP5 expression was investigated for the first time in patients with COVID-19. We reported serum OPN levels and IGFBP5 mRNA expressions in non-critically ill and critically ill patients. OPN and IGFBP5 levels may reflect disease severity and may be considered as independent risk factors. We have contributed to the discovery of markers that may aid in clinical decision-making and have the potential to be targets for future COVID-19 treatments. It may be beneficial for clinicians to consider OPN and IGFBP5 values when evaluating the status of COVID-19 patients.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

References

[1] Azkur AK, Akdis M, Azkur D, Sokolowska M, Veen W, Brüggen M, O'Mahony L, Gao Y, Nadeau K, Akdis CA. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 2020;75(7):1564–81. https://doi.org/10.1111/all.14364.
[2] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19). JAMA 2020;324(8):782–93. https://doi.org/10.1001/jama.2020.12839.
[3] Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med 2020;382(18):1708–20. https://doi.org/10.1056/NEJMoa2002032.
[4] Zou JN, Sun L, Wang BR, Zou Y, Xu S, Ding YJ, Shen LJ, Huang WC, Jiang XJ, Chen SM. The characteristics and evolution of pulmonary fibrosis in COVID-19 patients as assessed by AI-assisted chest HRCT. PLoS One 2021;16(3):e0248957. https://doi.org/10.1371/journal.pone.0248957.

[5] Güllü G, Karabulut S, Akkiprik M. Functional roles and clinical values of insulin-like growth factor-binding protein-5 in different types of cancers. Chin J Cancer 2012;31(6):266–80. https://doi.org/10.5732/cjc.111.10405.

[6] Skarlik C, Nezos A, Mavragani CP, Koutsilieris M. The role of insulin growth factors in autoimmune diseases. Ann. Res. Hosp 2019;3:310. https://doi.org/10.21037/ahr.2019.03.02. 10.

[7] Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23(6):824–54. https://doi.org/10.1210/er.2001-0033.

[8] Duan C, Xu Q. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. Gen Comp Endocrinol 2005;142(1–2):44–52. https://doi.org/10.1016/j.ygcen.2004.12.022.

[9] Duan C, Allard JB. Insulin-like growth factor binding protein-5 in physiology and disease. Front Endocrinol 2020;11:100. https://doi.org/10.3389/fendo.2020.00100.

[10] Pilewski JM, Liu L, Henry AC, Knauer AV, Feghali-Bostwick CA. Insulin-like growth factor proteins 3 and 5 are overexpressed in idiopathic pulmonary fibrosis and contribute to extracellular matrix deposition. Am J Pathol 2005;166(2):399–407. https://doi.org/10.1016/j.ajpath.2005.03.001.

[11] Nguyen XX, Muhammad L, Nietert PJ, Feghali-Bostwick C. IGFBP-5 promotes fibrosis via increasing its own expression and that of other pro-fibrotic mediators. Front Endocrinol 2018;9:601. https://doi.org/10.3389/fendo.2018.00601.

[12] Yanouka H, Yanaguchi Y, Feghali-Bostwick CA. The pro-fibrotic factor IGFBP-5 induces lung fibroblast and mononuclear cell migration. Am J Respir Cell Mol Biol 2009;41(2):179–88. https://doi.org/10.1165/rcmb.2008-0211OC.

[13] Clemente N, Raineri D, Cappellano G, Boggio E, Favero F, Soluri MF, et al. Osteopontin bridging innate and adaptive immunity in autoimmune diseases. J. Immunol. Res. 2016;2016:1–15. https://doi.org/10.1155/2016/7675437.

[14] Uede T. Osteopontin, intrinsic tissue regulator of intractable inflammatory diseases. Pathol Int 2011;61(5):265–80. https://doi.org/10.1111/j.1440-1827.2011.02649.x.

[15] Clemente N, Comi C, Raineri D, Cappellano G, Vecchio F, Orilieri E, et al. Role of anti-osteopontin antibodies in multiple sclerosis and experimental autoimmune encephalomyelitis. Front Immunol 2017;8:321. https://doi.org/10.3389/fimmu.2017.00321.

[16] Castello LM, Baldrighi M, Molinari I, Salmi I, Cantaluppi V, Vaschetto R, et al. The role of osteopontin as a diagnostic and prognostic biomarker in sepsis and septic shock. Cells 2019;8(2):174. https://doi.org/10.3390/cells8020174.

[17] Varm C, Demirci T, Cengiz H, Hacibekeoglu I, Tuncer FB, Çökletk E, et al. Relationship between serum osteopontin levels and the severity of COVID-19 infection. Wien Klin Wochenschr 2021;133(7–8):298–302. https://doi.org/10.1007/s00508-020-01789-5.

[18] Takahashi F, Takahashi K, Okazaki T, Maeda K, Ienaga H, Maeda M, et al. Role of osteopontin in the pathogenesis of bleomycin-induced pulmonary fibrosis. Am J Respir Cell Mol Biol 2001;24(3):264–71. https://doi.org/10.1165/ajrcmb.24.3.4293.

[19] Liu J, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 2001;25(4):402–8. https://doi.org/10.1016/j.meth.2001.12.002.

[20] Lang CH, Krawiec BJ, Huber D, McCoy JM, Frost RA. Sepsis and inflammatory insults downregulate IGFBP-5, but not IGFBP-4, in skeletal muscle via a TNF-dependent mechanism. Am J Physiol Regul Integr Comp Physiol 2006;290(4):R963–72. https://doi.org/10.1152/ajpregu.00684.2005.

[21] Vastrad B, Vastrad C, Tengli A. Bioinformatics analyses of significant genes, related pathways, and candidate diagnostic biomarkers and molecular targets in SARS-CoV-2/COVID-19. Gene Reports 2020;21:100956. https://doi.org/10.1016/j.gene.2020.100956.

[22] Hayek SS, Roderburg C, Blakely P, Launius C, Eugen-Olsen J, Tacke F, et al. Circulating osteopontin levels and outcomes in patients hospitalized for COVID-19. J Clin Med 2021;10(17):2907. https://doi.org/10.3390/jcm10172907.

[23] Reisner A, Blackwell LS, Sayeed I, Myers HE, Wali B, Heilman S, et al. Osteopontin as a biomarker for COVID-19 severity and multisystem inflammatory syndrome in children: a pilot study. Exp. Biol. Med. 2022;247(2):145–51. https://doi.org/10.1080/10899245.2021.1998617.

[24] Pardo A, Gibson K, Cisneros J, Richards TJ, Yang Y, Becerril C, et al. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. PLoS Med 2005;2(9):e251. https://doi.org/10.1371/journal.pmed.0020251.

[25] Nam TJ, Busby WH, Rees C, Clemmons DR. Thrombospondin and osteopontin bind to insulin-like growth factor (IGF)-Binding protein-5 leading to an alteration in IGF-I-stimulated cell growth. Endocrinology 2000;141(3):1100-6. https://doi.org/10.1210/endo.141.3.7386.

[26] Kim SK, Kwon JY, Nam TJ. Involvement of ligand occupancy in Insulin-like growth factor binding protein-5 decreases osteoblastic function in vitro. Bone 2004;35(5):54–60. https://doi.org/10.1016/j.bone.2004.08.011.