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Determination of both the expression and serum levels of epidermal growth factor and transforming growth factor β1 genes in COVID-19

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We aimed to evaluate the effects of both the expression and serum levels of *Epidermal growth factor* (*EGF*) and *Transforming growth factor-β1* (*TGF-β1*) genes in patients with different degrees of cellular damage as mild, moderate, severe, and critical illness that can lead to fibrosis caused by SARS-CoV-2. Totally 45 individuals (male: 21(46.67%); female: 24(53.33%)) with COVID-19 infection were included in this study. Four groups were constituted as mild (n = 16)], moderate (n = 10), severe (n = 10), and critical (n = 9) according to the severity of the disease. Blood samples were drawn from the patients, and all of the hemograms, *EGF* and *TGFβ1* gene expression, and serum levels were evaluated. The mean age of individuals was 57.311 ± 18.383 (min: 28, max: 94). Significant differences were found among the groups for PLT ($\chi^2 = 9.955$; $p = 0.019$), CRP ($\chi^2 = 7.693$; $p = 0.053$), Ferritin ($\chi^2 = 22.196$; $p < 0.001$), D-dimer ($\chi^2 = 21.982$; $p = 0.000$), LDH ($\chi^2 = 21.807$; $p < 0.001$) and all these parameters (exclude PLT in severe groups) was increased depending on the severity of the disease. Additionally, significant differences were detected for *EGF* ($\chi^2 = 29.528$; $p < 0.001$), *TGFB1* ($\chi^2 = 28.981$; $p < 0.001$) expression (that increased depending on the disease severity), and *EGF* ($\chi^2 = 7.84$; $p = 0.049$), *TGFB1* ($\chi^2 = 17.451$; $p = 0.001$) serum concentration levels (that decreased depending on the disease severity). This study found statistically significant differences for both *EGF* $2^{-\Delta\Delta Ct}$. *TGFβ1* $2^{-\Delta\Delta Ct}$ and *EGF*, *TGFβ1* serum concentration values among all patient groups. As disease severity increased, *EGF* $2^{-\Delta\Delta Ct}$. *TGFβ1* $2^{-\Delta\Delta Ct}$ levels increased, while *EGF* and *TGFβ1* serum concentration levels decreased. Perhaps this study will be useful in managing COVID-19 infection severity and pulmonary fibrosis cases secondary to COVID-19.

Keywords Cellular damage, Epidermal growth factor, İnfection, Pulmonary fibrosis, SARS-CoV-2, Transforming growth factor-β1

The rapid spread of coronavirus disease 19 (COVID-19) has prompted significant shifts within the medical and scientific communities in a remarkably short period. Despite the wealth of research on the epidemiology and immediate clinical symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, the long-term effects of this disease are only beginning to emerge[1]. While COVID-19 affects multiple systems, it primarily targets the lungs, leading to pneumonia and, in severe cases, acute respiratory distress syndrome (ARDS)[2–4]. Concerns about potential chronic pulmonary complications of COVID-19 infection, particularly pulmonary fibrosis, have been raised early in the pandemic due to its association with ARDS[5]. Recent studies indicate an increased risk of pulmonary fibrosis following severe COVID-19 infection, particularly among patients with comorbidities like diabetes, hypertension, or coronary disease. Furthermore, inflammation appears to contribute to permanent lung structural changes, including fibrosis[5–8].

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Transforming growth factor-β1 (*TGF-β1*) plays a central role in fibrogenesis, with the nucleocapsid protein of SARS-CoV-1 directly enhancing its activity. Given the similarity between the nucleocapsid proteins of SARS-CoV-2 and SARS-CoV-1, this mechanism may also contribute to lung fibrosis. Additionally, angiotensin II, which accumulates in the lungs due to virus-induced downregulation of *ACE-2*, upregulates *TGF-β1* along with connective tissue growth factor[9].

TGF-β1 activation stimulates fibroblasts to transform into myofibroblasts, driving fibrosis. Numerous studies have identified *TGF-β1* as a key pro-fibrotic factor that triggers Epithelial-mesenchymal transition (EMT) in pulmonary fibrosis, primarily through Smad-dependent or Smad-independent pathways[9–13].

Epidermal growth factor (*EGF*), a polypeptide growth factor, mediates its biological effects through the transmembrane protein *EGF* receptor (*EGFR*). It belongs to the group I *EGF* family, which includes *transforming growth factor-α* (*TGF-α*), *heparin-binding EGF (HB-EGF),* betacellulin, amphiregulin, epiregulin, and epigen[14].

Biological effects of *EGF* are mediated through the transmembrane protein *EGF* receptor (*EGFR*).

EGFR belongs to a structurally related family of tyrosine kinase receptors[15]. The *EGFR* signaling pathway is known to play a role in various processes, including inflammation and fibroblast proliferation in fibrosis[16].

Co-immunoprecipitation (co-IP) studies have provided significant insights into the interactions between Smad proteins and the *EGF* signaling pathway. These studies have demonstrated that *EGF* signaling can influence Smad activity, thereby affecting cellular processes such as proliferation and differentiation. Studies have indicated that Smad7, an inhibitory Smad, can interact with receptor-regulated Smads (R-Smads) upon *TGF-β* signaling. Co-IP experiments demonstrated that Smad7 oligomerizes with R-Smads, directly inhibiting their activity. This interaction serves as a negative feedback mechanism in the *TGF-β* signaling pathway, highlighting the complex regulatory roles of Smad proteins in cellular signaling. These findings underscore the intricate crosstalk between *EGF* and *TGF-β* signaling pathways mediated by Smad proteins, which play crucial roles in regulating cellular functions and disease progression[17,18].

We aimed to evaluate the effects of both the expression and serum levels of *EGF* and *TGF-β1* genes in patients with different degrees of cellular damage as mild, moderate, severe, and critical illness that can lead to fibrosis caused by SARS-CoV-2.

Methods

Patients and groups

Totally 45 individuals (male:21(46.67%); female:24(53.33%)) with COVID-19 infection were included in this study. Four groups were constituted as mild (n = 16)], moderate(n = 10), severe(n = 10), and critical(n = 9) according to the severity of the disease. The classification of the disease severity was performed according to the WHO classification system (WHO/2019-nCoV/clinical/2021.1). Pregnant women and individuals under 18 years were excluded from the study. None of our patients had received a COVID-19 vaccine.

The current study was approved by the Ministry of Health and Local Ethics Committee (Düzce University Local Ethic Committee, Date/Number 11.05.2020/89). All methods were carried out in accordance with relevant guidelines and regulations.

Detection of blood parameters and biomarkers

This study employed various laboratory techniques to detect several blood parameters and biomarkers. Hemogram parameters, including hemoglobin (HB), platelets (PLT), and lymphocytes, were analyzed using a Beckman Coulter LH 780 Analyzer. C-reactive protein (CRP) levels were determined using original commercial kits with a Roche Cobas Integra 400 plus autoanalyzer. D-dimer levels were measured using a Cobas Roche t511 analyzer, while a Roche Cobas 702 auto-analyzer was employed to measure serum LDH and ferritin levels.

The presence of SARS-CoV-2 was detected using a SARS-CoV-2 RTqPCR Detection Kit (Bioeksen, Turkey). Polymerase chain reactions (PCR) were performed using a Real-Time PCR analyzer (Anatolia Gene Works, Turkey).

Detection of human EGF and TGFB1 serum concentrations via enzyme-linked ımmunosorbent assay (ELISA)

Serum *EGF* and *TGFB1* levels of individuals participating in the study were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) based on biotin double antibody sandwich technology. This process utilized Human *EGF* and *TGFB1* kits from Bioassay Technology Laboratory.

Following each washing step with a Bio-Tek Instruments E.L.X. 50 Strip Washer, the optical density (OD value) of each well was measured using a 450 nm wavelength microplate reader (Bio-Tek Instruments ELX 800 Absorbance Microplate Reader). Results were obtained by substituting the absorbance values from the device into the calibration graph.

RNA ısolation and cDNA synthesis

RNA was isolated from peripheral blood samples of individuals using RiboEx (Catalog No: 301–001) and Hybrid-R (Catalog No: 305–101) kits, following the manufacturer's instructions. The extracted RNA was stored in RNase-free water at − 20 °C until further analysis. cDNA was synthesized from the isolated RNA using the WizScript™ cDNA Synthesis Kit (High Capacity) (Catalog No: W2211).

The reverse transcription reaction employed for cDNA synthesis followed these conditions: step 1 (25 °C for 10 min), step 2 (37 °C for 120 min), step 3 (85 °C for 5 min), and step 4 (4 °C, ~).

Relative gene expressions of EGF and TGFB1 gene by real-time qPCR

EGF, TGFB1, and the reference gene (ACTB) expression levels were determined for each cDNA sample using Applied Biosystems 7500 and ViiA7 Real-Time PCR Systems. The PCR was carried out using the WizPure™ 2X qPCR Master (SYBR) (Catalog No: W1711) kit in a final volume of 20 µL.

The Real-Time qPCR cycle conditions included an initial denaturation at 95 °C for 600 s, followed by 40 cycles of denaturation at 95 °C for 15 s, and 40 cycles of annealing at 60 °C for 60 s. ACTB served as the reference gene for quantifying mRNA expressions, which were normalized to the control group. Fold change calculations were performed by processing $^{\Delta\Delta Ct}$ values as $^{2\text{-}\Delta\Delta Ct}$.

Statistical analysis

The Statistical analysis was performed via the Statistical Package for Social Sciences (I.B.M. Corp., Armonk, NY, U.S.A.) for Windows 23.0. After the Shapiro–Wilk test, non-parametric tests for not normally distributed ($p < 0.05$) and parametric tests for normally distributed ($p > 0.05$) were carried out. The descriptive statistics [number, mean, standard deviation (S.D.), percentage, Mean Difference (I-J)] were performed for each variable. Homogeneity of variance test was detected to binary comparision of groups for Post-Hoc analaysis. While Tukey, Scheffe and LSD tests were performed on groups with equal variances, Tamhane's T^2 test was performed on groups without equal variances. Also, the Kruskal–Wallis and polynomial regression tests were performed to compare and correlate the groups. For statistical significance level, $p < 0.05$ was accepted.

Results

The mean age of individuals was 57.311 ± 18.383(min:28, max:94). Statistically significant differences were found among the groups for age, and the severity of the disease was increased depending on the age ($\chi^2 = 21.414$; $p < 0.001$). Significant differences were found among the groups for PLT ($\chi^2 = 9.955$; $p = 0.019$), CRP ($\chi^2 = 7.693$; $p = 0.053$), Ferritin ($\chi^2 = 22.196$; $p < 0.001$), D-dimer ($\chi^2 = 21.982$; $p = 0.000$), LDH ($\chi^2 = 21.807$; $p < 0.001$) and all these parameters (exclude PLT in severe groups) was increased depending on the severity of the disease. Also, significant differences were detected among the groups for SpO2 saturation that decreased depending on the severity of the disease ($\chi^2 = 39.389$; $p < 0.001$) (Table 1). Additionally, significant differences were detected for *EGF* ($\chi^2 = 29.528$; $p < 0.001$) (Table 1, Fig. 1b), *TGFB1* ($\chi^2 = 28.981$; $p < 0.001$) (Table 1, Fig. 2b) expression (that increased depending on the disease severity) and *EGF*($\chi^2 = 7.84$; $p = 0.049$) (Table 1, Fig. 1a,c), *TGFB1* ($\chi^2 = 17.451$; $p = 0.001$) (Table 1, Fig. 2a,c) serum concentration levels (that decreased depending on the disease severity).

When the binary comparison of the groups to be considered, statistically significant differences were detected between mild and moderate, mild and severe, mild and critical groups for ages ($p < 0.05$); between mild and severe, mild and critical, moderate and severe, moderate and critical for both *EGF* and *TGFB1* expression levels ($p < 0.05$); between mild and critical for *EGF* serum concentration levels ($p < 0.05$); between mild and severe, mild and critical for *TGFB1* serum concentration levels ($p < 0.05$); between mild and moderate, mild and severe, mild and critical, moderate and critical, severe and critical for Ferritin ($p < 0.05$). Also significant differences were detected between mild and critical, moderate and critical for D-dimer ($p < 0.05$); between mild and critical, moderate and critical, severe and critical for LDH($p < 0.05$); between mild and moderate, mild and severe, mild and critical, moderate and severe, moderate and critical, severe and critical for SpO2 ($p < 0.05$); between mild and moderate, mild and critical for CRP ($p < 0.05$) (Table 2).

	Groups				
	Mild (n = 16)	**Moderate (n = 10)**	**Severe (n = 10)**	**Critical (n = 9)**	**X^2/*p***
Age (years)	41.125 ± 15.297	57.3 ± 12.311	69.7 ± 11.373	72.333 ± 11.587	21.414/**0.000***
HB	12.994 ± 1.323	12.76 ± 1.974	12.1 ± 1.352	12.71 ± 1.02	3.271/0.352
PLT	201.5 ± 40.135	282.1 ± 132	310.3 ± 90,532	237.778 ± 127.694	9.955/**0.019***
Lymphocyte	687.5 ± 116.3	662 ± 309.365	590.4 ± 200.437	564 ± 240.269	2.866/0.413
CRP	2.6584 ± 1,884	6,928 ± 5.197	7.339 ± 8,349	8,14 ± 6.277	7.693/**0.053***
Ferritin	216.75 ± 61.623	497.5 ± 273.618	664 ± 421.937	1146.111 ± 470.134	22.196/**0.000***
D-dimer	0.359 ± 0.14	0.748 ± 0.544	1.156 ± 1.103	6.336 ± 7.114	21.982/**0.000***
LDH	311.688 ± 104.52	310.6 ± 89.077	361.4 ± 95.336	721.111 ± 220.898	21.807/**0.000***
SpO2	95.561 ± 1.151	90.3 ± 2.908	81.8 ± 5.181	68.3 ± 7.416	39.389/**0.000***
EGF $2^{-\Delta\Delta Ct}$	1.227 ± 0.8	1.661 ± 1.621	4.6 ± 2.259	7.827 ± 3.802	29.528/**0.000***
EGF conc	24.759 ± 15.329	17.052 ± 4.72	14.776 ± 6.059	13.314 ± 3.481	7.84/**0.049***
TGFβ1 $2^{-\Delta\Delta Ct}$	1.296 ± 1.136	1.927 ± 0.981	4.248 ± 2.345	10.28 ± 5.934	28.981/**0.000***
TGFβ1 conc	192.332 ± 213.327	129.772 ± 105.792	90.949 ± 21.962	68.561 ± 14.589	17.451/**0.001***

Table 1. The Hemogram values, *EGF* $2^{-\Delta\Delta Ct}$. *TGFβ1* $2^{-\Delta\Delta Ct}$ and *EGF, TGFβ1* concentration levels of each group. *EGF*: Epidermal Growth Factor; *TGFβ1:* Transforming Growth Factor Beta 1; Conc: Concentration; HB: Hemoglobin; PLT: Platelet; CRP: C-reactive protein; LDH: Lactate dehydrogenase; SpO2: Oxygen saturation level in the blood. *Statistically significant. Significant values are in [bold].

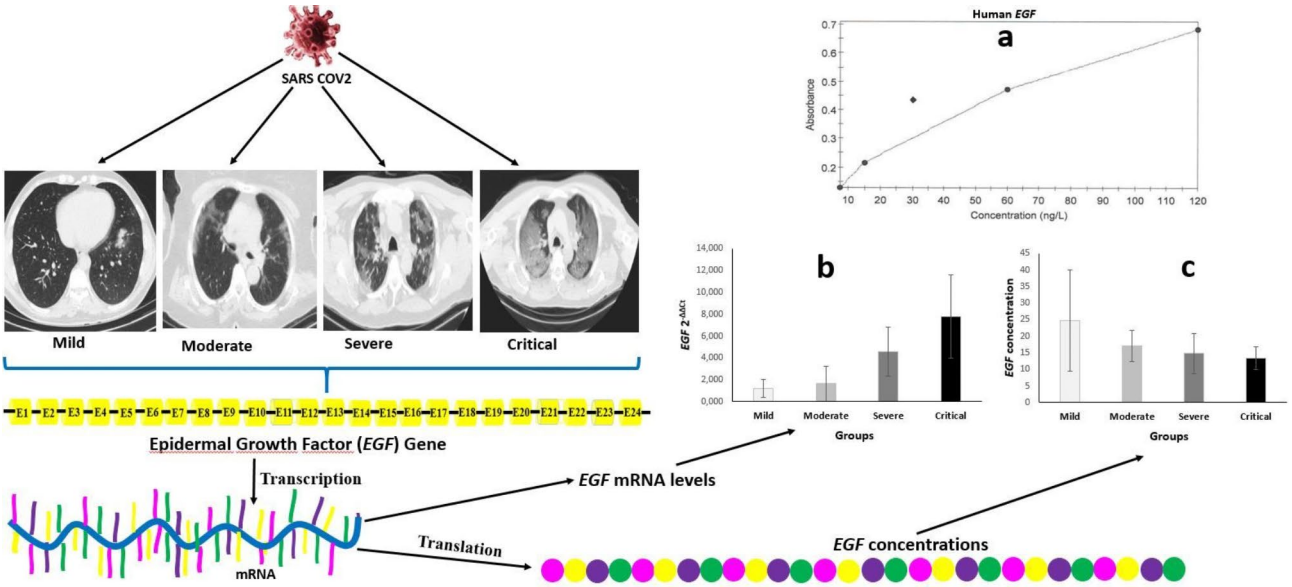

Fig. 1. Lung image of patients infected by SARS-COV2 grouped according to COVID-19 infection severity and both mRNA expression and serum concentration levels of the *EGF* gene in these patient groups. The patients were divided into four main groups: mild, moderate, severe, and critical, based on the severity of COVID-19 infection. Both $EGF^{2-\Delta\Delta Ct}$ and *EGF* serum concentration levels of patients were detected. After the COVID-19 infection, a cellular response occurs in the nucleus of cells. After the EGF mRNA is transcribed from the *EGF* gene, *EGF* proteins are formed from mature *EGF* mRNA. The ELISA standard curve is given in (**a**). There were significant differences among all groups for $EGF^{2-\Delta\Delta Ct}$ levels. As the severity of COVID-19 infection increased, the expression level of *the EGF* gene significantly increased, too. When the *EGF* gene expression levels were considered in the pairwise comparison, statistically significant differences were detected between mild and severe, mild and critical, moderate and severe, and moderate and critical groups (**b**). *EGF* serum concentration levels of groups are shown in (**c**). As the severity of COVID-19 infection increased, the serum *EGF* levels significantly decreased. A statistically significant difference was detected between mild and critical groups in terms of serum EGF concentration levels.

The differences were meaningful among the groups for additional disease ($\chi^2 = 17.117$; $p < 0.001$), hypertension ($\chi^2 = 13.46$; $p = 0.004$), chronic respiratory disease ($\chi^2 = 8.75$; $p = 0.033$), and last situation of the patients ($\chi^2 = 13.388$; $p = 0.004$) (Table 3).

Also, when the last situation (exitus/discharged) of the patients to be considered, statistically significant differences were not found for all of *EGF* expression levels ($\chi^2 = 45.000$; $p = 0.388$), *EGF* serum concentration levels ($\chi^2 = 39.939$; $p = 0.428$), *TGFB1* expression levels ($\chi^2 = 45.000$; $p = 0.430$), and *TGFB1* serum concentration levels ($\chi^2 = 39.938$; $p = 0.473$).

When the polynomial regression test was carried out, significant relation between *EGF* expression levels and age, *EGF* expression levels and CRP, *EGF* expression levels and Ferritin, *EGF* expression levels and D-dimer, *EGF* expression levels and LDH, *EGF* expression levels and SpO2, *EGF* expression levels and *EGF* serum concentration levels, *EGF* expression levels and *TGFB1* expression levels, *EGF* expression levels and *TGFB1* serum concentration levels, *EGF* serum concentration levels and age, *EGF* serum concentration levels and Hb, *EGF* serum concentration levels and Ferritin, *EGF* serum concentration levels and and SpO2, *EGF* serum concentration levels and *TGFB1* serum concentration levels ($p < 0.05$) (Table 4, Figs. 3, 4).

Additionally, there is significant relation between *TGFB1* expression levels and age, *TGFB1* expression levels and CRP, *TGFB1* expression levels and Ferritin, *TGFB1* expression levels and D-dimer, *TGFB1* expression levels and LDH, *TGFB1* expression levels and SpO2, *TGFB1* expression levels and *EGF* expression levels, *TGFB1* expression levels and *TGFB1* concentration levels, *TGFB1* serum concentration levels and age, *TGFB1* serum concentration levels and Hb, *TGFB1* serum concentration levels and CRP, *TGFB1* serum concentration levels and Ferritin, *TGFB1* serum concentration levels and LDH, *TGFB1* serum concentration levels and SpO2, *TGFB1* serum concentration levels and EGF serum concentration levels ($p < 0.05$) (Table 5, Figs. 5, 6).

Discussion

This study found a statistically significant difference for both *EGF* $2^{-\Delta\Delta Ct}$ *TGFβ1* $2^{-\Delta\Delta Ct}$ and *EGF, TGFβ1* serum concentration values among all patient groups. As disease severity increased, *EGF* $2^{-\Delta\Delta Ct}$, *TGFβ* $12^{-\Delta\Delta Ct}$ levels increased, while *EGF* and *TGFβ1* serum concentration levels decreased.

Research findings indicate that a decrease in hemoglobin[19,20] and lymphocyte levels[21,22] is associated with an increase in LDH[23,24], D-dimer[25–27], and ferritin[28,29] levels, correlating with the severity of the disease. Statistical analyses across all groups revealed significant differences for Ferritin ($p < 0.001$), D-dimer ($p < 0.001$), LDH ($p < 0.001$), and SpO2 ($p < 0001$). In our investigation, we observed a non-significant trend toward increased

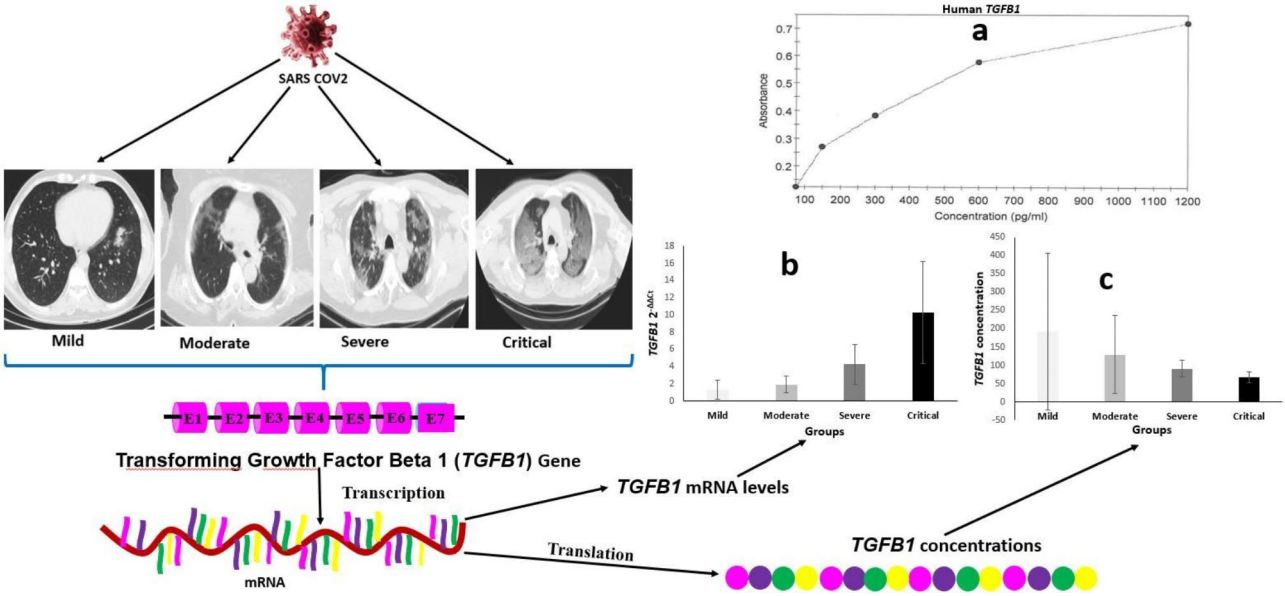

Fig. 2. Lung images of patients infected by SARS-COV-2 grouped according to COVID-19 infection severity and both mRNA expression and serum concentration levels of the *TGFβ1* gene in these patient groups. The patients were divided into four main groups: mild, moderate, severe, and critical, based on the severity of COVID-19 infection. Both $TGF\beta1^{2-\Delta\Delta Ct}$ and *TGFβ1* serum concentration levels of patients were detected. After the COVID-19 infection, a cellular response occurs in the nucleus of cells. After the *TGFβ1* mRNA is transcribed from the *TGFβ1* gene, *TGFβ1* proteins occur from mature *TGFβ1* mRNA. The ELISA standard curve is given in (**a**). There were significant differences among all groups for $TGF\beta1^{2-\Delta\Delta Ct}$ levels. As the severity of COVID-19 infection increased, the expression level of *the TGFβ1* gene significantly increased, too. When the *TGFβ1*gene expression levels were considered in the pairwise comparison, statistically significant differences were detected between mild and severe, mild and moderate, mild and critical, moderate and severe, moderate and critical, and severe and critical groups (**b**). *TGFβ1* serum concentration levels of groups are shown in (**c**). As the severity of COVID-19 infection increased, the serum *TGFβ1* levels significantly decreased. A statistically significant difference was detected between mild and severe, mild and critical, moderate and critical, and severe and critical groups in terms of serum *TGFβ1* concentration levels.

disease severity with decreasing lymphocyte values, while no statistically significant association was found with hemoglobin levels.

Cellular infection by SARS-CoV-2 relies on ACE-2 receptors, which also regulate the Renin-Angiotensin System (RAS). The virus has a preference for infecting type II pneumocytes due to their elevated ACE-2 expression compared to type I pneumocytes. However, the interaction between the S-Spike protein and ACE-2 can lead to ACE-2 downregulation, causing Ang II accumulation. This activates the ACE-AngII-AT1 axis, resulting in adverse effects such as vasoconstriction, inflammation, and fibrosis[30–32].

Ang II-induced collagen expression depends on *TGF-β1*, and increased Ang II activity usually leads to *TGF-β1* upregulation[33]. Despite studies investigating the correlation between *TGF-β1* and COVID-19 severity, findings remain inconclusive. *TGF-β1* influences immune cell development, differentiation, tolerance induction, and homeostasis, with pleiotropic effects contributing to either immune response or tolerance establishment. While previous research has implicated *TGF-β1* in various pathological processes, its role in COVID-19 progression and outcome remains inadequately explored[34].

COVID-19 infection can lead to a condition known as a "cytokine storm" in some patients, particularly in severe cases. This condition occurs as a result of an uncontrolled activation of the immune system and the release of excessive amounts of inflammatory cytokines. A cytokine storm can cause severe damage to the lungs, leading to acute respiratory distress syndrome (ARDS) and even death[35,36]. Key cytokines involved in the development of ARDS in COVID-19 infection[37]. TNF-α, IL-6, IL-1β, IFN-γ, and MCP-1, and measuring the levels of these cytokines may be useful in assessing disease severity and prognosis[35].

In this process, pro-inflammatory cytokines such as TNF-α play a significant role[38]. TNF-α can cause widespread inflammation in the body, leading to fluid accumulation in the lungs, alveolar collapse, and impaired gas exchange. This contributes to the development of ARDS. Additionally, TNF-α can increase vascular permeability, causing pulmonary edema and impaired oxygenation. Consequently, the cytokine storm and, in particular, the excessive production of TNF-α, is a critical factor in the development of ARDS and other serious complications in COVID-19 patients[35,36,39].

TGFB is a multifunctional cytokine that regulates cell growth and differentiation and activates various signaling pathways, including Smad-dependent and Smad-independent pathways. It plays a key role in the development of fibrosis, which is the accumulation of excess scar tissue in tissues, occurring through the

	Grps	Mild		Moderate		Severe		Critical	
		MD(I-J)	*p*	MD(I-J)	*p*	MD(I-J)	*p*	MD(I-J)	*p*
Age	Mild	–	–	**−16.175**	**0.042***	**−28.575**	**<0.001***	**−31.208**	**<0.001***
	Moderate	**−16.175**	**0.042***	–	–	**−12.400**	**0.173**	**−15.033**	**0.081**
	Severe	**−28.575**	**<0.001***	**−12.400**	**0.173**	–	–	**−2.643**	**0.997**
	Critical	**−31.208**	**<0.001***	**−15.033**	**0.081**	**−2.643**	**0.997**	–	–
EGF $2^{-\Delta\Delta Ct}$	Mild	–	–	−0.434	0,971	−3.373	**0.006***	−6.6	**0.005***
	Moderate	−0.434	0,971	–	–	−2.939	**0.024***	−6.166	**0.006***
	Severe	−3.373	**0.006***	−2.939	**0.024***	–	–	−3.227	**0.013***
	Critical	−6.6	**0.005***	−6.166	**0.006***	−3.227	**0.013***	–	–
EGF Conc	Mild	–	–	7.706	0.379	9.983	0.166	11.445	**0.044***
	Moderate	7.706	0.379	–	–	2.276	0.932	3.738	0.332
	Severe	9.983	0.166	2.276	0.932	–	–	−1.462	0.988
	Critical	11.445	**0.044***	3.738	0.332	−1.462	0.988	–	–
TGFB1 $2^{-\Delta\Delta Ct}$	Mild	–	–	−0.630	0.618	−2.952	**0.018***	−8.984	**0.011***
	Moderate	−0.630	0.618	–	–	0.99	**0.043***	−8.353	**0.017***
	Severe	−2.952	**0.018***	0.99	**0.043***	–	–	6.032	**<0.001***
	Critical	−8.984	**0.011***	−8.353	**0.017***	6.032	**<0.001***	–	–
TGFB1 Conc	Mild	–	–	62.56	0.27	101.363	**0.05***	123.77	**0.038***
	Moderate	62.56	0.27	–	–	38.823	0.535	61.21	0.343
	Severe	101.363	**0.05***	38.823	0.535	–	–	−23.387	**0.05***
	Critical	123.77	**0.038***	61.21	0.343	−23.387	**0.05***	–	–
Ferritin	Mild	–	–	−280.75	**0.05***	−447.25	**0.05***	−929.361	**0.002***
	Moderate	−280.75	**0.05***	–	–	−166.5	0.893	−648.611	**0.019***
	Severe	−447.25	**0.05***	−166.5	0.893	–	–	−482.11	**0.01***
	Critical	−929.361	**0.002***	−648.611	**0.019***	−482.11	**0.01***	–	–
D-dimer	Mild	–	–	−0.389	0.990	−0.796	0.928	−5.978	**<0.001***
	Moderate	−0.389	0.990	–	–	−0.408	0.992	−5.587	**0.003***
	Severe	−0.796	0.928	−0.408	0.992	–	–	−5.179	0.316
	Critical	−5.978	**<0.001***	−5.587	**0.003***	−5.179	0.316	–	–
LDH	Mild	–	–	1.087	1	−49.712	0.784	−409.423	**<0.001***
	Moderate	1.087	1	–	–	−50.8	0.873	−410.511	**<0001***
	Severe	−49.712	0.784	−50.8	0.873	–	–	−359.711	**<0.001***
	Critical	−409.423	**<0.001***	−410.511	**<0001***	−359.711	**<0.001***	–	–
SpO2	Mild	–	–	5.263	**0.001***	13.762	**<0001***	27.229	**<0001***
	Moderate	5.263	**0.001***	–	–	8.5	**0.003***	21.967	**<0001***
	Severe	13.762	**<0001***	8.5	**0.003***	–	–	13.467	**0.003***
	Critical	27.229	**<0001***	21.967	**<0001***	13.467	**0.003***	–	–
CRP	Mild	–	–	−4.269	0.05*	−4.680	0.166	−5.482	**0.021***
	Moderate	−4.269	0.05*	–	–	−0.411	0.998	−1.212	0.963
	Severe	−4.680	0.166	−0.411	0.998	–	–	−0.801	0.989
	Critical	−5.482	**0.021***	−1.212	0.963	−0.801	0.989	–	–
Platelet	Mild	–	–	−80.6	**0.046***	−108.8	**0.008***	−36.278	0.374
	Moderate	−80.6	**0.046***	–	–	−28.2	0.519	44.322	0.325
	Severe	−108.8	**0.008***	−28.2	0.519	–	–	72.522	0.111
	Critical	−36.278	0.374	44.322	0.325	72.522	0.111	–	–
Hb	Mild	–	–	0.233	0.979	0.894	0.437	0.283	0.966
	Moderate	0.233	0.979	–	–	0.66	0.744	0.049	1
	Severe	0.894	0.437	0.66	0.744	–	–	0.611	0.799
	Critical	0.283	0.966	0.049	1	0.611	0.799	–	–

Table 2. Binary comparison of the groups for *EGF* $2^{-\Delta\Delta Ct}$, *EGF, TGFB1* $2^{-\Delta\Delta Ct}$, *TGFB1 levels* and blood parameters. *EGF*: Epidermal Growth Factor; *TGFβ1:* Transforming Growth Factor Beta 1; Conc: Concentration; HB: Hemoglobin; CRP: C-reactive protein; LDH: Lactate dehydrogenase; SpO2: Oxygen saturation level in the blood; *: Statistically significant; MD(I-J): Mean Difference (I-J). Significant values are in [bold].

	Groups				
	Mild (n = 16)	Middle (n = 10)	Severe (n = 10)	Critical (n = 9)	X^2/p
Sex(M/F)	4(25%)/12(75%)	5(50%)/5(50%)	5(50%)/5(50%)	7(77.8%)/2(27.2%)	6.607/0.086
AD(Y/N)	4(125%)/12(75%)	8(80%)/2(20%)	9(90%)/1(10%)	8(88.9%)/1(11.1%)	17.117/**0.000***
DM(Y/N)	3(18.8%)/13(81.3%)	5(50%)/5(50%)	2(20%)/8(80%)	3(33.3%)/6(66.7%)	3.441/0.328
HT(Y/N)	2(12.5%)/14(87.5%)	5(50%)/5(50%)	2(20%)/8(80%)	3(33.3%)/6(66.7%)	13.46/**0.004***
CVA(Y/N)	0(0%)/16(100%)	1(10%)/9(90%)	0(0%)/10(100%)	2(22.2%)/7(77.8%)	5.536/0.137
CRD(Y/N)	0(0%)16(100%)	1(10%)/9(90%)	4(40%)/6(60%)	1(11.1%)/8(88.9%)	8.75/**0.033***
CRF(Y/N)	0(0%)16(100%)	1(10%)/9(90%)	0(0%)/10(100%)	0(0%)/9(100%)	3.58/0.311
Malig(Y/N)	0(0%)16(100%)	1(10%)/9(90%)	0(0%)/10(100%)	2(22.2%)/7(77.8%)	11.869/0.221
CVD(Y/N)	3(18.8%)/13(81.2%)	3(30%)/7(70%)	2(20%)/8(80%)	1(11.1%)/8(88.9%)	1.085/0.781
Last Sit(Ex/Dis)	0(0%)/16(100%)	1(10%)/9(90%)	0(0%)/10(100%)	4(44.4%)/5(55.6%)	13.388/**0.004***

Table 3. The sex, additional diseases and last situation of each group. M: Male; F: Female; AD: Additional Disease; Y: Yes; N: No; DM: Diabetes Mellitus; HT: Hypertension; CVA: Cerebrovascular accident; CRF: Chronic renal Failure; Malig: Malignancy; *: Statistically significant; CVD: Cardiovascular disease; CRD: Chronic respiratory disease; Sit: Situation; Ex:Exitus; Dis: discharged. Significant values are in [bold].

activation of *TGFB*/Smad signaling. *TGFB* drives this process by activating fibroblasts and increasing collagen production, which can lead to organ damage and dysfunction[40].

A study by Vaz de Paula CB et al.[34], revealed that COVID-19 patients exhibit more extensive diffuse alveolar damage and fibrosis in the alveolar septa compared to H1N1 patients. This was accompanied by a greater density of Collagen I and III, indicating a more pronounced fibrotic response. Notably, COVID-19 patients displayed elevated expression of several tissue biomarkers, including *ACE-2*, *AKT-1*, *CD44v6*, *IL-4*, *MMP-9*, *α-SMA*, *Sphingosine-1*, and *TGF-β1*. This heightened immunoexpression, particularly of *TGF-β1*, suggests a potential role of *TGF-β1* pathways in the development of pulmonary fibrosis associated with COVID-19.

In another study by Laloğlu et al.[41], *TGF-β1* serum levels increased with COVID-19 severity on the first and seventh days, but decreased by the seventh day in severe and critical patients. Karadeniz et al.[42] found no significant difference in *TGF-β1* concentrations between COVID-19 patients and healthy controls. Ghazavi et al.[43] reported higher serum *TGF-β1* concentrations among COVID-19 patients in a prospective case–control study. According to our results, *TGF-β1* gene expression begins to increase to respond to disease when the virus is encountered. It may be said that the severity of the disease probably decreases depending on the *TGF-β1* gene expression capacity increase. Although the *TGF-B1* gene expression level increases depending on the severity of the disease in order to respond to the disease, could the decrease in serum concentration levels be due to the decrease in the translation rate of *TGF-B1* mRNA due to the predominance of viral infection? Increasing *the TGF-B1* serum concentration level due to increased translation of *TGF-B1* mRNA may reduce the severity of the disease. To examine the daily change of *TGF-β1* level in other planned studies, It may be useful to explain changes in the pathogenesis of the disease.

Snezana Zivancevic-Simonovic and her colleagues[33] discovered that the concentration of *TGF-β1* was lower in individuals who succumbed to COVID-19 compared to those who survived. Furthermore, their correlation analysis revealed a robust positive connection between *TGF-β1* levels in patients' serum and platelet counts. Diminished serum levels of *TGF-β1* were linked to unfavorable outcomes in COVID-19 cases. They concluded that both *TGF-β1* levels and platelet counts exhibited a significant association with adverse disease outcomes in severely affected COVID-19 patients. In our investigation, we observed a decline in *TGF-β1* serum levels with increasing disease severity, coupled with elevated platelet values. This trend was noticeable across mild, moderate, and severe cases. Critically ill patients exhibited lower platelet values compared to those with severe disease. It's important to note that all severely ill patients in our study were undergoing steroid treatment, which could also impact platelet values; however, this aspect was not explored in the study data, representing a potential limitation.

The analysis of soluble cytokines and chemokines associated with COVID-19 ARDS and their correlation with mortality and disease progression has been extensively discussed. While the connection between *TGF-β1* and SARS-CoV-2 has been explored, the relationship between *EGF* and SARS-CoV-2 is less investigated. Previous suggestions indicate that acute lung injury triggers growth factor responses that initiate repair mechanisms for restoring lung integrity. *EGF*, in particular, plays a role in regulating bronchial and alveolar epithelial repair post-lung injury, enhancing lung fluid clearance by influencing the permeability of the alveolar epithelial junction. Furthermore, *EGF* gene polymorphisms have been associated with the risk of ARDS[44–47].

The Ras signaling pathway is a crucial signaling cascade that plays a role in cell growth, differentiation, and survival. This pathway is activated by various growth factors, including *EGF* and *TGFB*. Activation of the Ras signaling pathway leads to the activation and proliferation of cells called fibroblasts, which produce collagen and other proteins that provide structural support in tissues. However, excessive activation of the Ras signaling pathway can lead to the accumulation of excess collagen and a condition called fibrosis, which can cause organ damage and dysfunction. In particular, *EGF* and *TGF*-beta can promote fibrosis by activating the Ras signaling pathway and increasing collagen production in fibroblasts. Therefore, therapies that target the Ras signaling pathway may be a promising approach for treating fibrosis[48].

		Model summery					Parameter estimates			
Variable	***Equation***	R^2	***F***	***df1***	***df2***	***sig***	**Constant**	***b1***	***b2***	***b3***
EGF $2^{-\Delta\Delta Ct}$ and Age	Linear	.209	11.358	1	43	.002	48.780	2.514		
	Log									
	Cubic	.317	6.337	3	41	.001	44.288	4.287	.099	− .018
EGF $2^{-\Delta\Delta Ct}$ and PLT	Linear	.081	3.791	1	43	.058	220.953	8.809		
	Log									
	Cubic	.143	2.276	3	41	.094	230.624	12.047	− 2.388	.155
EGF $2^{-\Delta\Delta Ct}$ and CRP	Linear	.081	3.769	1	43	.059	4.071	.493		
	Log									
	Cubic	.086	1.287	3	41	.292	4.015	.331	.058	− .003
EGF $2^{-\Delta\Delta Ct}$ and Ferritin	Linear	.403	28.997	1	43	.000	269.386	86.946		
	Log									
	Cubic	.429	10.272	3	41	.000	217.881	104.126	2.099	− .254
EGF $2^{-\Delta\Delta Ct}$ and D-dimer	Linear	.387	27.091	1	43	.000	− .612	.716		
	Log									
	Cubic	.497	13.502	3	41	.000	.269	.427	− .037	.004
EGF Conc. and LDH	Linear	.180	9.419	1	43	.004	316.032	26.037		
	Log									
	Cubic	.228	4.028	3	41	.013	315.264	4.284	6.821	− .370
EGF $2^{-\Delta\Delta Ct}$ and SPO2	Linear	.587	61.002	1	43	.000	94.511	− 2.541		
	Logarithmic									
	Cubic	.618	22.078	3	41	.000	95.036	− 2.075	− .219	.013
EGF $2^{-\Delta\Delta Ct}$ and *EGF* Conc	Linear	.079	3.669	1	43	.062	21.628	− .910		
	Logarithmic									
	Cubic	.092	1.384	3	41	.261	22.761	− 1.539	.031	.001
EGF $2^{-\Delta\Delta Ct}$ and *TGFB1* $2^{-\Delta\Delta Ct}$	Linear	.706	103.029	1	43	.000	.088	1.120		
	Logarithmic									
	Cubic	.786	50.107	3	41	.000	1.123	− .314	.294	− .013
EGF $2^{-\Delta\Delta Ct}$ and *TGFB1* Conc	Linear	.051	2.301	1	43	.137	163.887	− 9.649		
	Logarithmic									
	Cubic	.079	1.175	3	41	.331	164.251	2.050	− 3.661	.199
EGF Conc. And Age	Linear	.266	15.596	1	43	.000	73.514	− .874		
	Logarithmic	.248	14.209	1	43	.000	112.008	− 19.536		
	Cubic	.297	5.776	3	41	.002	52.274	2.485	− .145	.002
EGF Conc. And Hb	Linear	.134	6.662	1	43	.013	11.779	.049		
	Logarithmic	.205	11.119	1	43	.002	8.762	1.402		
	Cubic	.220	3.853	3	41	.016	8.972	.379	− .010	8.705
EGF Conc. And PLT	Linear	.057	2.585	1	43	.115	292.925	− 2.270		
	Logarithmic	.071	3.296	1	43	.076	415.613	− 58.851		
	Cubic	.071	1.040	3	41	.385	354.172	− 8.770	.167	− .001
EGF Conc. And Ferritin	Linear	.085	4.004	1	43	.052	792.706	− 12.315		
	Logarithmic	.069	3.201	1	43	.081	1283.929	− 256.995		
	Cubic	.143	2.286	3	41	.093	− 55.425	116.456	− 5.361	.059
EGF Conc. And SPO2	Linear	.107	5.156	1	43	.028	79.690	.334		
	Logarithmic	.104	5.010	1	43	.030	64.503	7.638		
	Cubic	.133	2.088	3	41	.117	89.382	− 1.269	.072	− .001
EGF Conc. And *TGFB1* Conc	Linear	.318	20.078	1	43	.000	− 6.763	7.439		
	Logarithmic	.297	18.124	1	43	.000	− 333.995	166.135		
	Cubic	.489	13.087	3	41	.000	413.185	− 57.670	2.761	− .031

Table 4. Model summary and parameter estimates for between *both EGF* $2^{-\Delta\Delta Ct}$ and *EGF* concentration levels and hemogram parameters. *EGF*: Epidermal Growth Factor; *TGFβ1:* Transforming Growth Factor Beta 1; Conc: Concentration; HB: Hemoglobin; PLT: Platelet; LDH: Lactate dehydrogenase; SpO2: Oxygen saturation level in the blood; *: Statistically significant.

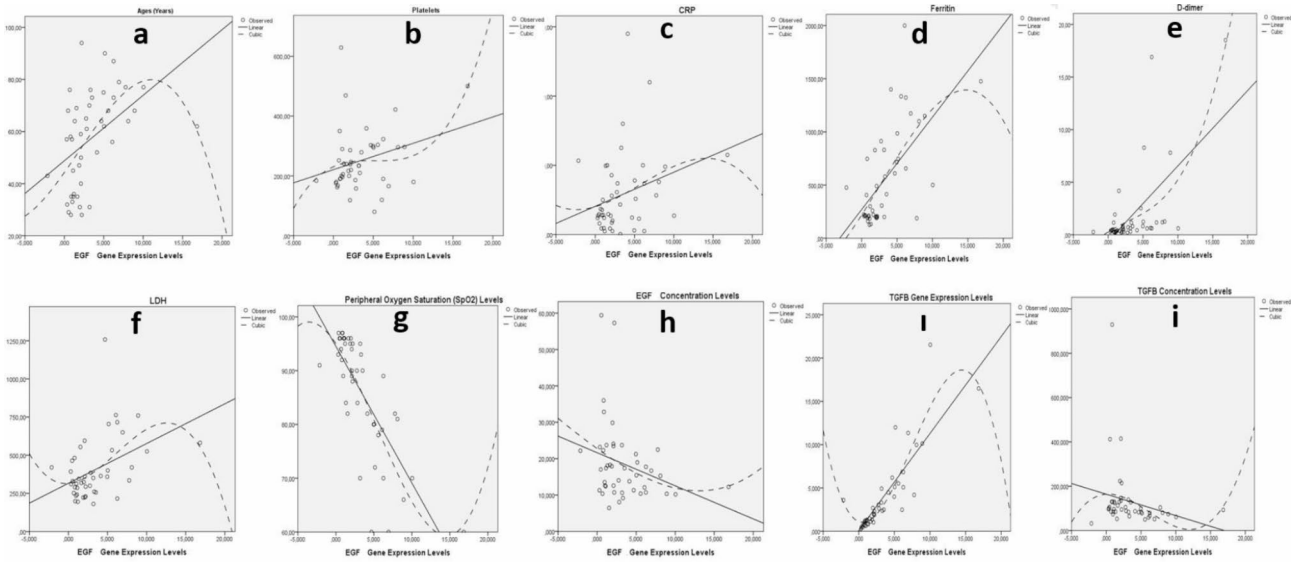

Fig. 3. Relationship between expression levels of *EGF* gene and other parameters. Statistically significant positive relationships were detected between *EGF* gene expression levels and ages (**a**), *EGF* gene expression levels and CRP (**c**), *EGF* gene expression levels and ferritin (**d**), *EGF* gene expression levels and D-dimer (**e**), *EGF* gene expression levels and LDH (**f**) and *EGF* gene expression levels and *TGFβ1* gene (**i**). Additionally, a statistically significant negative relationship between *EGF* gene expression levels and SpO2 (**g**) levels, *EGF* gene expression levels and EGF concentration levels (**h**), and *EGF* gene expression levels and *TGFβ1* concentration levels (**i**) were detected.

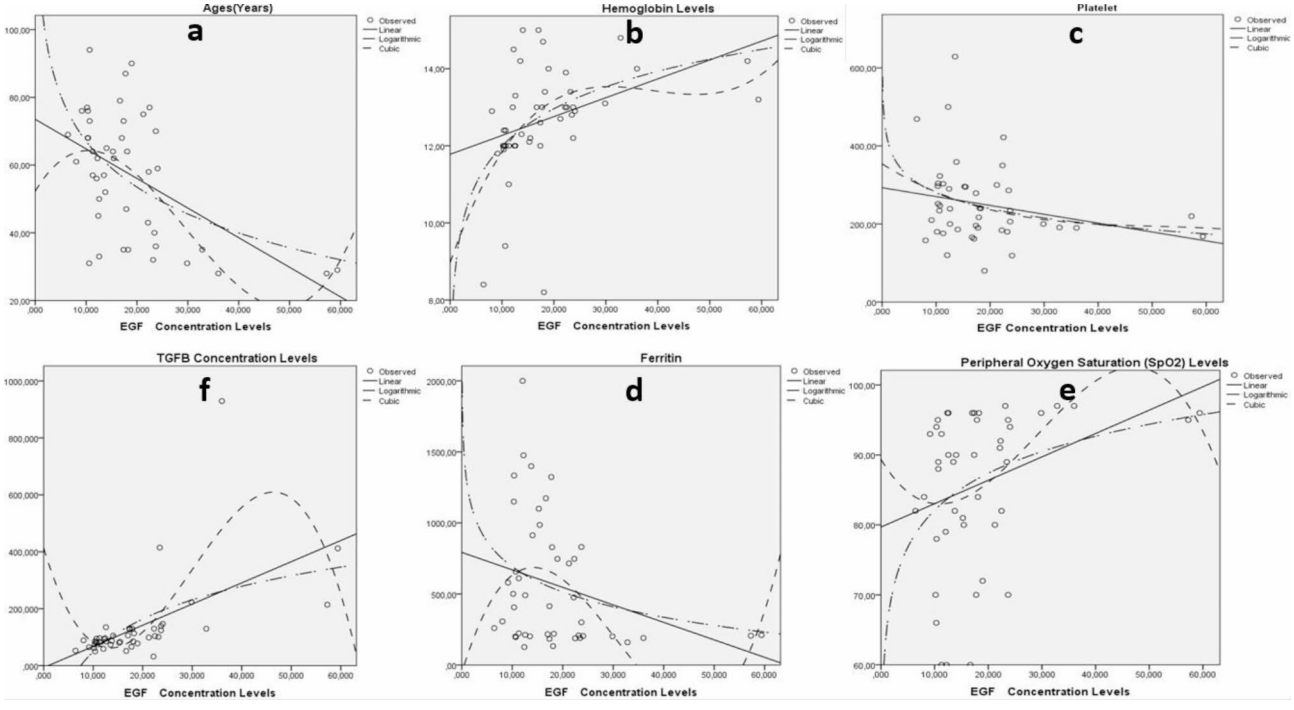

Fig. 4. Relationship between *EGF* concentration levels and other parameters. Statistically significant positive relationships were detected between *EGF* concentration levels and hemoglobin (**b**), *EGF* concentration levels and *TGFβ1* concentration levels (**f**), and *EGF* concentration levels and SpO2 levels (**e**). A statistically significant negative relationship between *EGF* concentration levels and ages (**a**) and *EGF* concentration levels and *Ferritin* (**d**) were also detected.

		Model summery					Parameter estimates			
Variable	***Equation***	***R^2***	***F***	***df1***	***df2***	***sig***	**Constant**	***b1***	***b2***	***b3***
TGFB1 $2^{-\Delta\Delta Ct}$ and Age	Linear	.191	10.173	1	43	.003	50.296	1.804		
	Log	.235	13.203	1	43	.001	50.067	8.609		
	Cubic	.256	4.700	3	41	.007	42.837	6.449	− .439	.010
TGFB1 $2^{-\Delta\Delta Ct}$ and PLT	Linear	.005	.214	1	43	.646	244.493	1.633		
	Log	.010	.453	1	43	.505	242.259	10.203		
	Cubic	.013	.173	3	41	.914	240.718	1.821	.411	− .024
TGFB1 $2^{-\Delta\Delta Ct}$ and CRP	Linear	.060	2.749	1	43	.105	4.502	.319		
	Log	.107	5.154	1	43	.028	4.200	1.835		
	Cubic	.142	2.254	3	41	.096	3.335	.716	.028	− .003
TGFB1 $2^{-\Delta\Delta Ct}$ and Ferritin	Linear	.237	13.348	1	43	.001	369.973	49.996		
	Log	.360	24.188	1	43	.000	341.041	265.451		
	Cubic	.422	9.993	3	41	.000	143.890	167.698	− 6.542	− .012
TGFB1 $2^{-\Delta\Delta Ct}$ and D-dimer	Linear	.266	15.621	1	43	.000	.084	.446		
	Log	.207	11.258	1	43	.002	.392	1.695		
	Cubic	.450	11.164	3	41	.000	1.393	− .935	.242	− .009
TGFB1 Conc. and LDH	Linear	.207	11.239	1	43	.002	322.850	20.965		
	Log	.201	10.828	1	43	.002	329.522	88.962		
	Cubic	.290	5.594	3	41	.003	291.283	27.313	2.037	− .134
TGFB1 $2^{-\Delta\Delta Ct}$ and SPO2	Linear	.574	57.929	1	43	.000	93.218	− 1.885		
	Logarithmic	.593	62.635	1	43	.000	92.831	− 8.251		
	Cubic	.684	29.514	3	41	.000	96.765	− 3.531	.044	.003
TGFB1 $2^{-\Delta\Delta Ct}$ and *EGF* $2^{-\Delta\Delta Ct}$	Linear	.706	103.029	1	43	.000	.944	.630		
	Logarithmic	.645	78.048	1	43	.000	1.211	2.593		
	Cubic	.742	39.227	3	41	.000	.510	.772	.012	− .001
TGFB1 $2^{-\Delta\Delta Ct}$ and *TGFB* Conc	Linear	.058	2.647	1	43	.111	161.204	− 7.729		
	Logarithmic	.098	4.696	1	43	.036	167.647	− 43.380		
	Cubic	.107	1.637	3	41	.196	218.789	− 46.488	4.234	− .112
TGFB1 Conc. And Age	Linear	.199	10.655	1	43	.002	64.820	− .057		
	Logarithmic	.268	15.760	1	43	.000	133.101	− 16.326		
	Cubic	.303	5.955	3	41	.002	84.946	− .327	.001	− 3.754
TGFB1 Conc. And Hb	Linear	.049	2.233	1	43	.142	12.391	.002		
	Logarithmic	.090	4.238	1	43	.046	9.229	.745		
	Cubic	.129	2.016	3	41	.127	10.590	.029	− 7.848	5.530
TGFB Conc. And PLT	Linear	.019	.828	1	43	.368	263.880	− .099		
	Logarithmic	.021	.926	1	43	.341	370.396	− 25.753		
	Cubic	.027	.376	3	41	.771	301.008	− .624	.001	− 9.361
TGFB1 Conc. And CRP	Linear	.033	1.484	1	43	.230	6.715	− .007		
	Logarithmic	.090	4.242	1	43	.046	19.590	− 2.983		
	Cubic	.128	2.014	3	41	.127	14.407	− .119	.000	− 2.176
TGFB1 Conc. And Ferritin	Linear	.079	3.693	1	43	.061	682.425	− .900		
	Logarithmic	.157	8.017	1	43	.007	2009.323	− 311.256		
	Cubic	.192	3.257	3	41	.031	1295.675	− 9.520	.023	− 1.500
TGFB1 Conc. And LDH	Linear	.035	1.562	1	43	.218	439.605	− .269		
	Logarithmic	.093	4.414	1	43	.042	902.982	− 107.406		
	Cubic	.149	2.399	3	41	.082	742.681	− 4.701	.013	− 8.942
TGFB1 Conc. And SPO2	Linear	.090	4.248	1	43	.045	82.841	.023		
	Logarithmic	.174	9.038	1	43	.004	49.099	7.925		
	Cubic	.231	4.110	3	41	.012	65.176	.277	− .001	4.844
TGFB1 Conc. And *EGF* Conc	Linear	.318	20.078	1	43	.000	12.927	.043		
	Logarithmic	.450	35.228	1	43	.000	-39.430	12.487		
	Cubic	.548	16.574	3	41	.000	.478	.193	.000	8.787

Table 5. Model summary and parameter estimates for between *both TGFB1* $2^{-\Delta\Delta Ct}$ and *TGFB1* concentration levels and hemogram parameters. *EGF*: Epidermal Growth Factor; *TGFβ1:* Transforming Growth Factor Beta 1; Conc: Concentration; HB: Hemoglobin; PLT: Platelet; CRP: C-reactive protein; LDH: Lactate dehydrogenase; SpO2: Oxygen saturation level in the blood; *: Statistically significant.

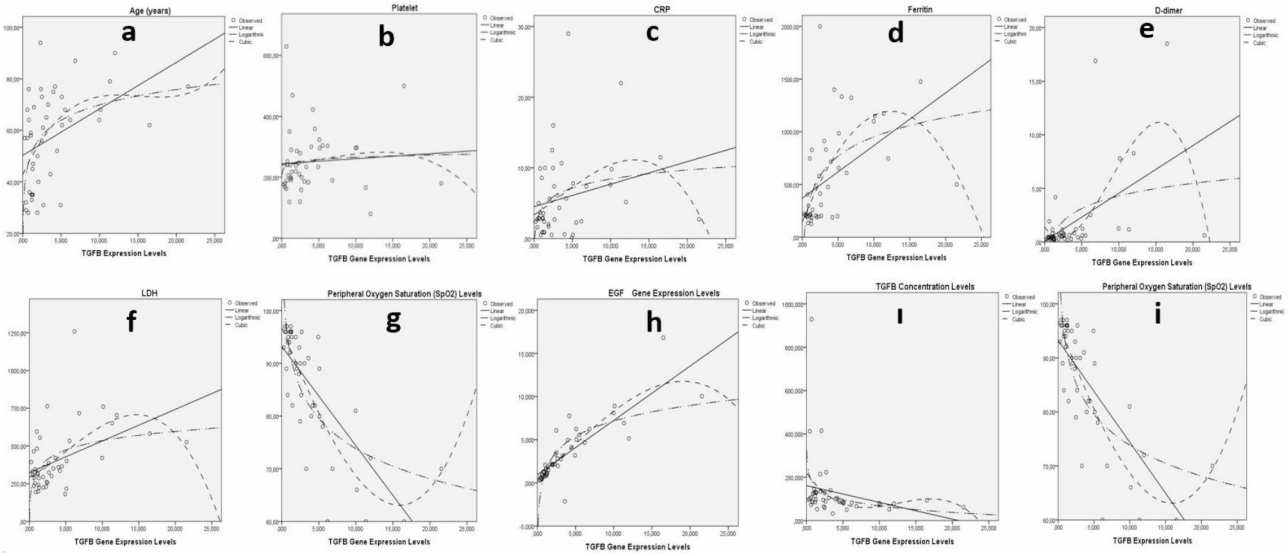

Fig. 5. Relationship between expression levels of *TGFβ1* gene and other parameters. Statistically significant positive relationships were detected between *TGFβ1* gene expression levels and ages (**a**), *TGFβ1* gene expression levels and CRP (**c**), *TGFβ1* gene expression levels and ferritin (**d**), *TGFβ1* gene expression levels and D-dimer (**e**). *TGFβ1* gene expression levels and LDH (**f**) and *TGFβ1* gene expression levels and *EGF* gene (**h**). Additionally, statistically significant negative relationship between *TGFβ1* gene expression levels and SpO2 (**g**) levels, *TGFβ1* gene expression levels and *TGFβ1* concentration levels (**i**), and *TGFβ1* concentration levels and SpO2 levels (**i**) were detected.

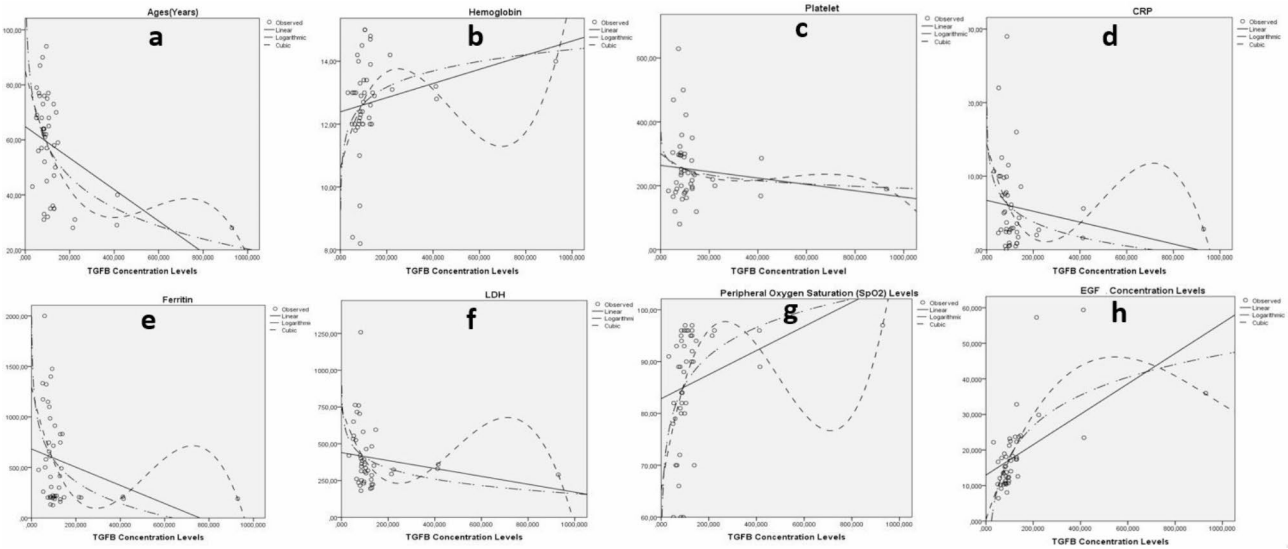

Fig. 6. Relationship between *TGFβ1* concentration levels and other parameters. Statistically significant positive relationships were detected between *TGFβ1* concentration levels and hemoglobin (**b**), *TGFβ1* concentration levels and SpO2 levels (**g**), and *TGFβ1* concentration levels and *EGF* concentration levels (**h**). Additionally, statistically significant negative relationship between *TGFβ1* concentration levels and ages (a), *TGFβ1* concentration levels and CRP (**d**), *TGFβ1* concentration levels and *Ferritin* (**e**) and *TGFβ1* concentration levels and LDH (**f**) were detected.

In a study by Balnis et al.[49] that evaluated 41 patients who needed mechanical ventilators due to severe ARDS, Decreased *EGF* levels were associated with mortality on day 45. The strength of our study was that we also looked at *EGF* expression levels. Our study found that *EGF* gene expression levels increased depending on disease severity, whereas *EGF* serum levels decreased as disease severity increased.

Our study revealed that *EGF* gene expression increases with disease severity, while serum *EGF* levels decrease. This suggests that although *EGF* expression may rise in response to infection and lung damage, the decreased

translation of *EGF* mRNA results in insufficient *EGF* to prevent severe disease. However, it is important to note that other studies have reported conflicting results, highlighting the need for further research in this area.

There are studies on *EGF* levels that contradict our study. In the study conducted by Marija Petrushevska and her colleagues[50] in 14 severe COVID-19 infections and 20 control groups, They found *EGF* levels to be higher in patients with COVID-19 infection. In this study, since they do not determine the *EGF* level according to disease severity, it will be difficult to explain its place in disease pathogenesis. It is likely that they will find lower *EGF* levels in healthy individuals, but reporting the correlation with severity will contribute more to pathogenesis.

Our study has some limitations. First, it is a single center with a limited number of patients. Not examining our patients' treatment is among our study's limitations. One limitation of our study is that *EGF*/*TGF-β1* levels were only measured at a single time point. A longitudinal study design is necessary to better understand the changes in these factors over time and their association with fibrosis development. Also; our another limitation is during the peak of the COVID-19 pandemic, many studies faced challenges in recruiting healthy control groups due to strict lockdown measures and ethical considerations. Consequently, researchers often categorized patients based on disease severity for analysis. For instance, a study by Ayouni et al.[51]. evaluated public health interventions during the pandemic, noting that stringent measures limited the inclusion of healthy controls, leading to analyses focused on patient subgroups. Similarly, a systematic review by Franco et al.[52] assessed post-COVID-19 conditions, highlighting difficulties in obtaining control groups and emphasizing analyses based on patient severity classifications.

In conclusion**,** considering the prevalence and characteristics of the COVID-19 epidemic, it is highly likely that SARS-CoV-2 will become an endemic virus. Vaccines have proven to be effective agents in controlling the disease, but on the other hand, there are now serious concerns about post-COVID-19 sequelae, especially those related to pulmonary fibrosis. This study is planned to shed light on further studies to reveal the effects of *EGF* and *TGF-β1* on COVID-19-related fibrosis and the severity of the disease. However, more in-depth research is needed to understand these mechanisms fully. In particular, future studies focusing on the interactions of these factors at the cellular level and their roles in specific signaling pathways may further increase knowledge in this field. Our findings showing that *EGF* and *TGF-β1* levels influence the severity of COVID-19 disease highlight the potential of these factors to modulate the course of infection. In this context, understanding the effects of these factors on clinical outcomes and disease course may be important in guiding future treatment strategies.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
This study was approved by the Ministry of Health and Local Ethics Committee (Düzce University Local Ethic Committee, Date/Number 11.05.2020/89).

Consent to participate
All patients have signed the informed consent.

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