ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Relationship of Serum Beta Defensin-2 and Surfactant Protein A and B Levels with the Clinical Course and Prognosis of COVID-19 Infection

Serum Beta Defensin-2 ve Sürfaktan Protein A ve B Düzeylerinin COVID-19 Enfeksiyonunun Klinik Seyri ve Prognozu ile İlişkisi

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ABSTRACT

Aim: *Defensin and surfactant-related peptides are antimicrobial peptides that play an essential role in the natural host defense against micro-organisms. The objective is to compare the serum beta-defensin 2 (ß-def2), serum surfactant protein-A (sSPA) and B (sSPB) levels and respiratory surfactant protein A (rSPA) and B (rSPB) levels in patients with clinically mild and severe COVID-19 pneumonia.*

Material and Method: *On presentation at the hospital before any treatment, venous blood samples and a nasopharyngeal smear sample were taken for rSPA and rSPB. The ß-def2, SPA, and SPB levels were advanced and analyzed using the ELISA method.*

Results: *The levels of acute phase reactants of ß-def2, sSPA, sSPB and white blood cell (WBC), neutrophil count, ferritin, procalcitonin, and C-reactive protein (CRP) were determined to be higher in the clinically and radiologically severe patients. The rSPA and rSPB levels showed a tendency to be lower in this patient group but not to a statistically significant level. The ß-def2, sSPA and sSPB values were determined to be positively correlated with WBC, neutrophil count, NLR, ferritin, procalcitonin, and CRP levels and negatively correlated with the albumin level.*

Conclusion: *ß-def2, sSPA, and sSPB, which play a role in the natural host defense, are correlated with the acute phase reactants of the clinical and radiological severity of COVID-19:WBC, neutrophil count, NLR, ferritin, procalcitonin, and CRP. In patients with severe disease, rSPA and rSPB levels tended to be low, although not statistically significant, and further studies on this subject could guide the use of surfactants in treatment.*

Keywords: *beta defensin-2; COVID-19; surfactant protein A; surfactant protein B*

ÖZET

Amaç: *Defensin ve sürfaktan, mikroorganizmalara karşı doğal konak savunmasında önemli rol oynayan antimikrobiyal peptitlerdir. Bu çalışmanın amacı, klinik olarak hafif ve şiddetli COVID-19 pnömonisi olan hastalarda serum beta-defensin2 (ß-def2), serum sürfaktan protein-A (sSPA) ve B (sSPB) seviyeleri ile respiratuvar sürfaktan protein A (rSPA) ve B (rSPB) seviyelerini karşılaştırmaktır.*

Materyal ve Metot: *Herhangi bir tedaviye başlamadan önce hastaneye başvurduklarında venöz kan örnekleri alındı ve rSPA ve rSPB için nazofarengeal sürüntü örneği alındı. ß-def2, SPA ve SPB seviyelerinin ileri analizi ELISA yöntemiyle yapıldı.*

Bulgular: *Akut faz reaktanları olan ß-def2, sSPA, sSPB ve beyaz kan hücresi (WBC), nötrofil sayımı, ferritin, prokalsitonin ve C-reaktif protein (CRP) seviyelerinin, klinik ve radyolojik olarak şiddetli hastalarda daha yüksek olduğu belirlendi. rSPA ve rSPB seviyelerinin bu hasta grubunda istatistiksel olarak anlamlı olmayacak şekilde daha düşük olduğu eğilimi gösterdi. ß-def2, sSPA ve sSPB değerleri, WBC, nötrofil sayımı, NLR, ferritin, prokalsitonin ve CRP seviyeleri ile pozitif korelasyon gösterirken albümin seviyesi ile negatif korelasyon gösterdiği belirlendi.*

Sonuç: *Doğal konak savunmasında rol oynayan ß-def2, sSPA, sSPB, WBC, nötrofil sayımı, NLR, ferritin, prokalsitonin ve CRP gibi klinik ve radyolojik şiddetin akut faz reaktanları ile korele olup, şiddetli hastalığı olan hastalarda rSPA ve rSPB seviyelerinin istatistiksel olarak anlamlı olmasa da düşük olduğu eğilimindedir ve bu konu üzerinde daha fazla çalışma, tedavide surfactant kullanımı için rehberlik edebilir.*

Anahtar kelimeler: *beta defensin-2, COVID-19, yüzey aktif madde protein A, yüzey aktif madde protein B*

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Introduction

Antimicrobial peptides (AMP) in the mucosa, a component of primary host defense, are produced by epithelial cells and cells in the natural immune system and play a role as modulators of the natural immune system in the defense against infections[1]. Defensin is an AMP found in many organisms, including mammals, insects, and plants. In mammals, β-defensins are produced by epithelial cells and leukocytes, then stored as bioactive molecules in neutrophils in circulation[2]. These peptides have an essential role in the fight against infections caused by bacteria and viruses such as Haemophilus influenzae by activating immature dendritic cells[3,4]. They also show a chemotactic effect for monocytes, polymorphonuclear leukocytes and T-cells, and strengthen the acquired immune response[5,6].

The capability of a virus to resist natural antiviral immunity is related to the pathogenicity of the virus, which affects the severity of the disease[7]. Cytokines such as Type 1 interferon (IFN) play an essential role in the natural antiviral immune response by regulating immune cells and producing proteins[8]. However, some viruses, including Middle East respiratory syndrome coronavirus (MERS-CoV), inhibit the induction pathways of cytokines such as type 1 IFN[9]. This is related to immune avoidance mechanisms such as T-cell inactivation with the downregulation of antigen presentation and the induction of macrophage apoptosis[10].

Surfactant proteins, such as serum surfactant protein-A (SPA) and Surfactant protein D (SPD), found within pulmonary surfactant in the lungs, have a role in host defense and the regulation of inflammation[11,12]. Previous studies have suggested that SPA plays a role in regulating hBD3 (human β defensin 3)-mediated activation of mast cells[13,14]. Defensins and surfactant proteins are essential in early host defense against gram-positive and gram-negative bacteria, mycobacteria, and fungi. Moreover, several studies have documented the activities of the antiviral characteristics of defensins against both enveloped and non-enveloped viruses[5].

Acute phase reactants (APR) are a heterogenous plasma protein group that increases or decreases in response to inflammatory stimuli such as infections, trauma, acute arthritis, systemic autoimmune disorders and neoplasms[15]. The production of APRs in response to infection is stimulated by the liver and mediated by proinflammatory cytokines produced by macrophages, monocytes, and other cells participating in the inflammatory response. C-reactive protein (CRP), procalcitonin (PCT), and serum ferritin are among the most essential APRs. The albumin level decreases as a response to inflammation and is therefore known as a negative APR[16].

The novel coronavirus disease (COVID-19) emerged in Wuhan, China, in December 2019 and, with its rapid spread across the world, was declared a global pandemic within a few months as a disease primarily affecting the respiratory system and lungs and causing thousands of deaths[17]. Although there are several studies in literature related to COVID-19, to the best of our knowledge, no study has examined defensin and surfactant levels. This study aimed to compare the serum beta-defensin2 (ß-def2), serum surfactant protein A (sSPA) and B (sSPB) levels and respiratory surfactant protein A (rSPA) and B (rSPB) levels on nasopharyngeal swabs in patients with clinically mild COVID-19 and patients with clinically severe COVID-19 pneumonia. The comparisons of these parameters aimed to determine their role in the clinical course and prognosis of COVID-19 and contribute to the understanding of the disease course.

Materials and Methods

The Ethics approved this study of Committees of the University of Health Sciences, Bakirköy Dr. Sadi Konuk Training and Research Hospital (approval number: 2020/197) and conducted by the principles of the Declaration of Helsinki and its later amendments or comparable ethical standards.

The hospital Ethics Committee granted approval for this observational, descriptive, cross-sectional study and all procedures were applied in compliance with the principles of the Helsinki Declaration. The study included patients diagnosed with COVID-19 with positivity in Real-Time Polymerase Chain Reaction (RT-PCR) analysis of nasal and pharyngeal smear samples, who were admitted and followed up in the hospital between 01.06.2020 and 01.08.2020. Patients were excluded from the study if they were aged <18 years, did not consent to participate in the study, received immunosuppressive treatment, had congenital immune suppression, or had received anti-inflammatory or mucolytic treatment within the previous 15 days.

At the time of presentation at the hospital, before administering any antiviral or immune suppressive treatment, including steroids, a 1 cc venous blood sample was withdrawn into a yellow-top tube and nasal and pharyngeal smear samples were taken. The venous blood sample was centrifuged and then stored at -80°C, and the smear samples were stored directly. Analysis of the

ß-def2 (DEFB2 ELISA kit KTE62104, Abbkine), SPA (SP-A ELISA kit KTE60675 Abbkine) and SPB (SP-B ELISA kit KTE60674 Abbkine) levels was performed in the advanced analysis laboratory of the hospital with the ELISA (Enzyme-Linked Immunosorbent Assay) method. In the ELISA method, microplates provided in the kits are pre-coated with antibodies specific to the protein sought. First, standards and samples were added, and then protein-specific biotin-conjugated antibody was added to the appropriate microplate wells. Avidin conjugated to Horseradish Peroxidase (HRP) was then added to each microplate well and incubated. After the substrate solution was added, only the wells containing the sought protein-specific biotin-conjugated antibody and enzyme-conjugated Avidin showed a color change. The enzyme-substrate reaction was terminated by adding sulfuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450nm ±10nm. Protein concentrations in the samples were then evaluated by comparing sample O. D. s to the standard curve.

In the hospital laboratory, the reference ranges were taken as 3.7–10.1/μm^3 for leukocytes, 1.63–6.96 μm^3 for neutrophils, 1.09–2.99 μm^3 for lymphocytes, 12.9–15.9 g/dl for hemoglobin, and 35–52 g/l for albumin. The upper limit for PCT was defined as 0.5ng/ml, and for CRP, 5 mg/l. Internal quality control and external quality reliability were applied to ensure the accuracy of the tests. For the albumin, CRP, and ferritin levels, a Beckman Coulter AU5800 clinical chemistry analyzer was used (Beckman Coulter, Brea, CA, USA). An ADVIA 2120 hematology autoanalyzer (Siemens Healthcare Diagnostics, Erlangen, Germany) was used for the complete blood count (CBC).

For the classification of lung involvement of the patients, a semi-quantitative scoring system was used on the thoracic computed tomography (CT) images. Each of the five lobes of the lungs was scored from 0–5 as follows: 0:no involvement, 1: <5% involvement, 2:25% involvement, 3:26%-49% involvement, 4:50%-74% involvement, 5: >75% involvement[18]. In this study, the patients were separated into three groups: mild involvement (<25%), moderate level involvement (26–74%), and severe involvement (>75%).

The patients were grouped according to the clinical condition as mild, moderate, and severe. Patients with mild symptoms and oxygen saturation within normal limits (>98%) were accepted as mild disease. Patients with clinical or radiographic evidence of lower respiratory tract disease and blood oxygen saturation ≥94% in room air were accepted as moderate disease. The signs of severe disease were accepted as evident tachypnea (respiratory rate ≥30 breaths per min), hypoxemia (oxygen saturation ≤93%, breathed oxygen fraction rate of partial arterial oxygen pressure <300), and pulmonary leakage (>50% involved lung area within 24–48 hours)[19].

Statistical Analysis

Data obtained in the study were analyzed statistically using Number Cruncher Statistical System vn—2007 software (NCSS, Kaysville, Utah, USA). In comparing two groups of quantitative data showing normal distribution, the student's t-test was applied; for data not showing normal distribution, the Mann-Whitney U-test was used. The One-Way ANOVA test was applied to comparisons of three or more groups showing normal distribution, and the Bonferroni test was applied to paired comparisons. For data not conforming to a normal distribution, the Kruskal Wallis test was applied to three groups or more comparisons, and the Bonferroni Dunn test to paired comparisons. Spearman's correlation analysis was used in the evaluation of relationships between variables. A value of $p<0.05$ was accepted as statistically significant.

Results

Evaluation was made of 94 patients in this prospective study, comprising 57 (60.6%) males and 37 (39.4%) females with a mean age of 53 years (range 19–86 years). Demographic and clinical characteristics are given in Table 1. The WBC, neutrophil, NLR, ferritin, PCT,

Table 1. *Demographic and clinical characteristics of the patients*

		n	%
Age (year)	*Min-Max (Median)*	19–86 (55)	
	Mean ± SD	53.18±15.14	
Gender	Female	37	39.4
	Male	57	60.6
Comorbid diseases	Diabetes Mellitus	27	28.7
	Hypertension	28	29.8
	CAD	12	12.8
	COPD	12	12.8
	Others	4	4.3
Clinical condition	Mild	48	51.1
	Moderate + Severe	46	48.9
Thoracic CT	Mild	29	30.9
	Moderate	33	35.1
	Severe	32	34
Stay in ICU	Non	86	91.5
	Yes	8	8.5
Length of hospital stay (days)	*Min-Max (Median)*	3–50 (9)	
	Mean ± SD	12.21±9.63	
Survival status	Survivor	88	93.6
	Nonsurvivor	6	6.4

• Multiple diseases are seen.
CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, CT: computed tomography, ICU: intensive care unit

and CRP values were higher in the patients with severe thoracic CT findings than those with mild and moderate CT findings. The lymphocyte and albumin values were lower in the patients with severe thoracic CT findings than those with mild and moderate CT findings. In the patients with moderate and severe clinical status, the lymphocyte and albumin measurements were lower (p=0.001), and the neutrophil, NLR (p=0.001), ferritin, PCT, and CRP values (p<0.01) were determined to be higher than those of clinically mild patients (Table 2).

When the patients were grouped according to clinical severity, the ß-def2, sSPA and sSPB measurements in the moderate and severe groups were statistically significantly higher than those of the clinically mild patients (p=0.017, p=0.044, respectively). No difference was determined between the groups of clinical severity with respect to the rSPA and rSPB measurements (p>0.05) (Table 3).

A statistically significant difference was determined between the cases according to thoracic CT findings in respect of β-def2 measurements. The β-def2 values of the patients with severe thoracic CT findings were higher than those of patients with mild and moderate findings (p=0.001).

The sSPA measurements were statistically significantly higher in the cases with severe CT findings compared

Table 2. *Laboratory findings according to CT involvement and clinical condition*

		Thoracic CT				Clinical condition		
		Mild (n=29)	Moderate (n=33)	Severe (n=32)	*p*	Mild (n=48)	Moderate + Severe (n=46)	*p*
WBC (per µm³)	***Min-Max (Median)***	3.1–9.7 (5.5)	2.6–8.3 (5.7)	2.8–15.6 (6.8)	[a]***0.020****	3.1–9.7 (5.5)	2.6–15.6 (6)	[c]***0.068***
	Mean ± SD	5.50±1.51	5.58±1.53	7.66±3.51		5.60±1.54	6.96±3.20	
Lymphocyte (per µm³)	***Min-Max (Median)***	0.8–2.7 (1.6)	0.7–3 (1.4)	0.4–3.1 (1.1)	[a]***0.001****	0.8–3 (1.6)	0.4–3.1 (1.1)	[c]***0.001****
	Mean ± SD	1.62±0.56	1.54±0.57	1.11±0.50		1.64±0.58	1.18±0.50	
Neutrophil (per µm³)	***Min-Max (Median)***	0.7–7.4 (3.2)	1.5–5.7 (3.4)	1.6–14.1 (5.2)	[a]***0.001****	0.7–7.4 (3.2)	1.5–14.1 (4.5)	[c]***0.001****
	Mean ± SD	3.31±1.42	3.50±1.19	5.98±3.36		3.39±1.34	5.22±3.08	
NLR	***Min-Max (Median)***	0.3–9.3 (2)	0.9–5.2 (2.2)	1.8–33.3 (4.9)	[a]***0.001****	0.3–9.3 (2)	1.2–33.3 (3.9)	[c]***0.001****
	Mean ± SD	2.45±1.84	2.52±1.15	6.70±6.75		2.40±1.56	5.51±5.92	
Albumin (g/L)	***Min-Max (Median)***	27.6–48.3 (40.6)	15.2–46 (38.9)	27.8–45 (36)	[b]***0.001****	15.2–48.3 (39.8)	27.8–45 (36.3)	[d]***0.001****
	Mean ± SD	40.59±4.22	38.15±5.07	36.14±3.72		39.61±5.22	36.76±3.58	
Ferritin (µg/L)	***Min-Max (Median)***	3–674 (108)	6.5–1499 (177)	22.4–1631(376.5)	[a]***0.002****	3–1070 (105.5)	22.4–1631 (359.5)	[c]***0.001****
	Mean ± SD	164.26±161.83	283.28±350.41	400.04±335.15		161.60±179.49	416.44±363.66	
PCT (ng/ml)	***Min-Max (Median)***	0–0.4 (0)	0–0.7 (0.1)	0–6.4 (0.1)	[a]***0.001****	0–0.4 (0)	0–6.4 (0.1)	[c]***0.001****
	Mean ± SD	0.07±0.08	0.09±0.12	0.40±1.11		0.06±0.06	0.32±0.93	
CRP (mg/dl)	***Min-Max (Median)***	1–165 (12)	2–148 (27)	14–375 (98.5)	[a]***0.001****	1–165 (16.4)	6.1–375 (83.5)	[c]***0.001****
	Mean ± SD	34.28±41.62	36.99±35.97	116.68±77.89		30.99±34.69	96.97±75.66	

[a]Kruskal-Wallis Test, [b]Oneway ANOVA Test, [c]Mann-Whitney U Test, [d]Student t Test; *p<0.05, **p<0.01.
WBC: white blood cells, NLR: neutrophil to lymphocyte ratio, PCT: procalcitonin, CRP: C-reactive protein.

Table 3. *Comparison of parameters according to clinical condition*

		Clinical condition		*p*
		Mild (n=48)	Moderate + severe (n=46)	
ß-def2 (pg/ml)	***Min-Max (Median)***	9.4–149.8 (29.3)	9.4–130 (43.2)	***0.017****
	Mean ± SD	36.06±23.6	57.42±38.27	
sSPA (pmol/L)	***Min-Max (Median)***	11.8–202.7 (43.1)	18.2–122.8 (52.8)	***0.044****
	Mean ± SD	48.40±28.91	57.23±25.52	
sSPB (ng/L)	***Min-Max (Median)***	65.2–448.9 (159.7)	58.3–457.1 (223.7)	***0.009****
	Mean ± SD	165.73±64.8	230.95±113.28	
rSPA (pmol/L)	***Min-Max (Median)***	0.7–41.4 (13.5)	0.4–39.6 (11.7)	***0.846***
	Mean ± SD	16.65±12.6	15.53±11.17	
rSPB (ng/L)	***Min-Max (Median)***	24.4–135.5 (57.5)	22.7–134.4 (56.5)	***0.676***
	Mean ± SD	70.18±32.19	65.32±29.45	

Mann-Whitney U Test, *p<0.05, **p<0.01.
ß-def2:beta-defensin2, sSPA: serum surfactant protein A, sSPB: serum surfactant protein B, rSPA: respiratory surfactant proteinA, rSPB: respiratory surfactant proteinB.

Table 4. *Comparison of parameters according to thoracic CT involvement*

		Thoracic CT			*p*
		Mild (n=29)	**Moderate (n=33)**	**Severe (n=32)**	
ß-def2 (pg/ml)	***Min-Max (Median)***	15.1–149.8 (32.8)	9.4–92.7 (26.9)	10.4–130 (61.7)	***0.001****
	Mean ± SD	40.69±26.3	32.39±19.59	67.28±40.75	
sSPA (pmol/L)	***Min-Max (Median)***	19–202.7 (46.3)	17.1–82.3 (41.7)	11.8–122.8 (59.5)	***0.005****
	Mean ± SD	55.23±33.35	41.72±15.96	61.94±28.07	
sSPB (ng/L)	***Min-Max (Median)***	79.1–448.9 (175.2)	58.3–245.8 (142.8)	85.5–457.1 (284.9)	***0.001****
	Mean ± SD	184.57±75.84	146.05±47.08	263.74±115.55	
rSPA (pmol/L)	***Min-Max (Median)***	1.2–39.6 (14.4)	0.4–35.9 (11.6)	0.7–41.4 (9.5)	***0.656***
	Mean ± SD	16.64±10.42	15.81±12.02	15.8±13.35	
rSPB (ng/L)	***Min-Max (Median)***	24.4–135.5 (60.4)	22.7–134.4 (57.5)	33–129 (45.3)	***0.447***
	Mean ± SD	71.36±30.95	66.91±32.36	64.89±29.93	

Kruskal-Wallis Test *p<0.05, **p<0.01.
CT: computed tomography, ß-def2:beta-defensin2, sSPA: serum surfactant protein A, sSPB: serum surfactant protein B, rSPA: respiratory surfactant proteinA, rSPB: respiratory surfactant protein B.

Table 5. *Relationship between parameters and laboratory findings*

		ß-def2 (pg/ml)	**sSPA (pmol/L)**	**sSPB (ng/L)**	**rSPA (pmol/L)**	**rSPB (ng/L)**
WBC (per µm^3)	r	0.245	0.276	0.312	-0.035	-0.014
	p	***0.021****	***0.008*****	***0.002*****	***0.745***	***0.897***
Lymphocyte (per µm^3)	r	-0.072	-0.057	-0.106	-0.101	0.022
	p	***0.505***	***0.585***	***0.312***	***0.338***	***0.835***
Neutrophil (per µm^3)	r	0.293	0.271	0.341	0.019	-0.013
	p	0.005**	0.009**	0.001**	0.860	0.904
NLR	r	0.264	0.242	0.322	0.090	-0.013
	p	***0.013****	***0.020****	***0.002*****	***0.394***	***0.902***
PLT	r	0.120	-0.114	-0.115	0.152	0.245
	p	***0.264***	***0.283***	***0.273***	***0.145***	***0.018****
Albumin (g/L)	r	-0.399	-0.243	-0.284	-0.003	0.046
	p	0.001**	0.019*	***0.006*****	***0.976***	***0.661***
Ferritin (µg/L)	r	0.148	0.159	0.158	0.069	0.191
	p	***0.167***	***0.128***	***0.130***	***0.518***	***0.068***
PCT (ng/ml)	r	0.244	0.250	0.256	0.061	0.111
	p	***0.021****	***0.016****	***0.013****	***0.568***	***0.292***
CRP (mg/dl)	r	0.328	0.205	0.307	-0.005	-0.074
	p	0.002**	0.049*	0.003**	0.966	0.485

r: Spearman's correlation coefficient *p<0.05, **p<0.01.
ß-def2:beta-defensin2, sSPA: serum surfactant protein A, sSPB: serum surfactant protein B, rSPA: respiratory surfactant proteinA, rSPB: respiratory surfactant proteinB, WBC: white blood cells, NLR: neutrophil to lymphocyte ratio, PLT: platelet, PCT: procalcitonin, CRP: C-reactive protein.

to those with moderate CT findings (p=0.004). The sSPB measurements of the group with severe CT findings were higher than those of the patients with mild and moderate CT findings (p=0.044, p=0.001, respectively).

According to the thoracic CT findings, no statistically significant difference was determined between the cases in respect of the rSPA and rSPB measurements (p>0.05) (Table 4).

A positive very weak correlation was determined between the ß-def2 measurement and the WBC and PCT measurements (r=0.245; r=0.244; p<0.05), and a positive weak correlation between the ß-def2 measurement and neutrophil, NLR, and CRP values (r=0.293; r=0.264; r=0.328; p<0.05). A negative, weak correlation was determined between the ß-def2 measurement and the albumin value (r=-0.399; p<0.05).

The sSPA measurement was determined to be very weakly positively correlated with the NLR, PCT, and CRP values (r=0.242; r=0.250; r=0.205; p<0.05), weakly positively correlated with the WBC and neutrophil values (r=0.276; r=0.271; p<0.05), and very weakly negatively correlated with the albumin measurement (r=-0.243; p<0.05).

A weak positive correlation was determined between the sSPB measurement and the WBC, neutrophil, NLR, and CRP values (r=0.312; r=0.341; r=0.322; r=0.307; p<0.05), and a very weak positive correlation was determined between sSPB and the PLT and PCT values (r=0.245; r=0.256; p<0.05). There was determined to be a very weak negative correlation between sSPB and the albumin value (r=-0.284; p<0.05). No significant correlation was detected between ß-def2, sSPA, sSPB, rSPA, rSPB measurements and lymphocyte and ferritin levels (Table 5).

Discussion

The results of this prospective study demonstrated a positive correlation between elevated levels of natural immunity proteins (β-def2, sSPA, sSPB) and the known positive acute phase reactants of CRP, PCT, and ferritin in COVID-19 patients defined as clinically and radiologically severe at the time of first presentation at the hospital before the administration of any treatment which could affect the immune system, immune response, or the production of surfactant.

The cytokine storm is known to play a significant role in the clinical status of COVID-19. The development of a cytokine storm is a potentially fatal immune condition characterized by the over-production of more than 150 inflammatory cytokines and chemical mediators expressed by immune or non-immune cells and the rapid proliferation and hyperactivation of T-cells, macrophages, and natural killer cells[20]. Increasing serum levels of ferritin, PCT, and CRP, known to be proinflammatory molecules and correlate with infection severity, were seen in the current study to be statistically significantly higher in patients with a more severe clinical and radiological disease course. Albumin, accepted as a negative acute phase reactant, was significantly lower in the clinically and radiologically more severe COVID-19 patients. As shown in several previous studies, the lymphocyte ratio was lower in the current study of patients classified clinically and radiologically as more severe[21,22].

When an organism faces an endogenous or exogenous threat, the first line of defense is natural immunity[23]. Previous studies have shown that defensins are antibacterial effectors of the natural immune response and function as antiviral peptides[24]. The antibacterial mechanism of antimicrobial peptides, such as defensin, depends on the pathogen being rendered inactive through disrupting the pathogen membrane stability by cationic peptides. In one of the first studies to show this, Daher et al. suggested that the ability of defensin to directly inactivate HSV and other enveloped viruses, including influenza A virus, could be due to the ability of defensin to destabilize viral envelopes[25]. In a study by Kerget et al., which examined the role of defensin in COVID-19, the alpha-defensin level in COVID-19 patients with pulmonary involvement and clinical acute respiratory distress syndrome (ARDS) was higher than in healthy individuals[26]. ARDS is a life-threatening lung injury that allows fluid to leak into the lungs. In the current study, the β-def2 level was significantly higher in clinically severe patients and those with severe radiological involvement.

SPD, a part of the natural immune system, is one of the collectin protein families synthesised by Type 2 alveolar epithelium. In addition to SPD, SPA and SPB target alveolar macrophages, dendritic cells, and T-cells and play an essential role in agglutination, optimisation, and modulation. Previous studies have shown that serum SPD levels increase with disease severity in patients infected with SARS-CoV, a coronavirus similar to that responsible for severe acute respiratory syndrome (SARS)[27–31]. Similarly, in the current study, sSPA and sSPB levels were determined to be high in patients with more severe disease, both clinically and radiologically. In ARDS, there is known to be a disruption in lung surfactant activity and a reduction in content and components of active large surfactant aggregates[32]. In COVID-19 with pulmonary involvement, there is consumption of surfactant with ARDS, the formation of hyalin membrane[33], together with virus-origin lysis of Type II pneumocytes, the formation of ground-glass opacities and bilateral infiltrates radiographically, reduced pulmonary compliance and refractory hypoxemia occurs[34]. Surfactant activity deficiencies can be weakened with an increase in active surfactant concentration[35]. The appropriate administration of exogenous surfactant has proven effective in premature infants with ARDS[36]. There are recommendations for the use of surfactants in the treatment of COVID-19, especially in patients who have developed clinically severe ARDS[37].

In the current study, rSPA and rSPB tended to be lower, although not at a level of statistical significance, in clinically severe patients and those with severe radiological involvement. The reason for this could be that the alveolar surfactant level was examined at the time of diagnosis, that no sample was taken when the

clinical status of ARDS progressed and that samples taken were not entirely from the alveoli (a method such as bronchoalveolar lavage was not used).

This study had some limitations, primarily that samples were only taken on presentation before administering any medication or treatment that could affect cytokines and blood levels. Therefore, changes in the parameters examined at advanced stages of the disease were unknown. One of the limitations of our study was that there was no COVID-negative control group. The whole world has been affected by COVID-19 for nearly two years, and for new developments in the treatment and prevention of COVID-19, real-life data would be of help in determining the pathways which play a role in the pathogenesis of the disease. Therefore, there is a need for furthermore extensive studies in this area.

In conclusion, the results of this study demonstrated that ß-def2, sSPA, and sSPB values were significantly higher in clinically severe patients and those with severe pulmonary involvement. In patients with severe disease, rSPA and rSPB levels showed a tendency to be lower, although not statistically significant. When it is considered that the serum surfactant level is low in an ARDS table and it is even recommended to administer surfactant to patients in treatment, that the rSPA and rSPB values were not statistically significantly low could be a sign that they could fall further in advanced stages of the disease.

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Authors' Contributions

Constructing an idea or hypothesis for the research and literature review: GSE, NI; Designing and planning methodology: GSE, NI; Providing material and environmental supports and data collection: RK, KKY, MEI, PK, TST; Analysis and interpretation: GSE, NI, PK, SKT, TST; Writer, supervision and critical review: GSE, PK, TST, RK, MEI, SKT, KKY, NI.

Conflict of Interest

We declare that there is no conflict of interest.

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