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Angiotensin (1−7) peptide replacement therapy with plasma transfusion in COVID-19

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ABSTRACT

Aim: To determine whether convalescent angiotensin (1−7) peptide replacement therapy with plasma (peptide plasma) transfusion can be beneficial in the treatment of critically ill patients with severe coronavirus 2 (SARS-CoV-2) infection.

Study design: Case series of 9 critically ill patients with laboratory-confirmed COVID-19 who met the following criteria: severe pneumonia with rapid progression and continuously high viral load despite antiviral treatment. Peptide plasma: Plasma with angiotensin (1−7) content 8–10 times higher than healthy plasma donors was obtained from suitable donors. Peptide plasma transfusion was applied to 9 patients whose clinical status and/or laboratory profile deteriorated and who needed intensive care for 2 days.

Results: In our COVID-19 cases, favipiravir, low molecular weight heparin treatment, which is included in the treatment protocol of the ministry of health, was started. Nine patients with oxygen saturation of 93% and below despite nasal oxygen support, whose clinical and/or laboratory deteriorated, were identified. The youngest of the cases was 36 years old, and the oldest patient was 85 years old. 6 of the 9 cases had male gender. 3 cases had been smoking for more than 10 years. 4 cases had at least one chronic disease.

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In all of our cases, SARS CoV2 lung involvement was bilateral and peptide plasma therapy was administered in cases when oxygen saturation was 93% and below despite nasal oxygen support of 5 liters/minute and above, and intensive care was required. Although it was not reflected in the laboratory parameters in the early period, 8 patients whose saturations improved with treatment were discharged without the need for intensive care. However, a similar response was not obtained in one case. Oxygen requirement increased gradually and, he died in intensive care process. An increase of the platelet count was observed in all cases following the peptide plasma treatment.

Conclusion: In this preliminary case series of 9 critically ill patients with COVID-19, administration of plasma containing angiotensin (1−7) was followed by improvement in their clinical status. The limited sample size and study design preclude a definitive statement about the potential effectiveness of this treatment, and these observations require evaluation in clinical trials.

1. Introduction

The renin-angiotensin system (RAS) is a hormone system that regulates blood pressure, fluid and electrolyte balance, as well as systemic vascular resistance [1]. While vasoconstriction, proliferation, and inflammation occur via the renin / Angiotensin 1 (AT1) / Angiotensin II pathway (inflammation producing pathway) via the AT1 receptor, angiotensin 1 and 2 are degraded by the angiotensin-converting enzyme 2 (ACE2) / Angiotensin (1−7) pathway (anti-inflammatory) and Anijotensin (1−7) (Ang-(1−7)) is created. Ang-(1−7) acts in the opposite way of Angiotensin II (Ang II), binding to Mas receptors, causing vasodilation, antiproliferative and anti-inflammatory effects.

Chronic disease state and advanced age are important risk factors for Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) infection [2]. In chronic diseases, the RAS is overactivated by the effect of either disease and advanced age. The RAS has two arms working opposite to each other. The ACE-2/Ang-(1−7) /Mas receptor pathway creates a protective balance between Ang II and Ang-(1−7) in chronic diseases in which the RAS system is overactive. Normal or lower blood pressure is observed during pregnancy despite RAS activation due to the hormones [3]. The reason for the decreased vascular response to Ang-II in pregnant women is considered to be related to the higher levels of Ang (1−7) and its balancing effects on vascular tone [4]. Supporting this, preeclamptic women who had hypertension related to pregnancy demonstrated higher Ang (1−7) levels than normal pregnants [5,6]. ACE inhibitors (ACE-I) prolonge the half-life of Ang (1−7) up to 3 h resulting in a 5–25 fold increase in peptide levels in the blood [6–8]. Besides, Ang (1−7) levels are reported to be enhanced up to 20 fold in accordance with the gestational week in pregnancy [3].

SARS-CoV-2 causes loss of ACE-2 receptor activity leads to less Ang II, inactivation, and less Ang-(1−7) formation [7]. Understanding the ACE-2/Ang-(1−7)/Mas receptor pathway can be a key to improving the resolution of inflammation and attenuating pro-inflammatory responses by limiting inflammatory tissue damage and disease [8]. Ang-(1−7) level is 5–20 times higher in individuals who have advanced age (over 50 years old), have chronic diseases such as diabetes, chronic obstructive pulmonary disease (COPD), asthma [9], exposure to ACE inhibitor/ARB for at least 3 years [10–12], smoking [13,14] have not had the Novel Coronavirus Disease 2019 (COVID-19) than in healthy persons/individuals [9–14]. Diffuse pulmonary inflammation, endothelial inflammation, and thrombosis are key features of COVID-19. SARS-CoV-2 binds to ACE-2 (angiotensin-converting enzyme 2) receptors and enters the cell via fusion of the cell membrane. Loss of ACE-2 receptor activity from the outer region of the membrane leads to less angiotensin II inactivation and less angiotensin1–7 formation. In several experimental models of lung injury, an imbalance between angiotensin II (overactivity) and antiotensin (1−7) (deficiency) was shown to trigger inflammation, thrombosis, and other adverse reactions. Angiotensin II / Ang-(1−7) imbalance can be important in the progression of SARS CoV2 infection. In this view, some therapeutic approaches, including plasma containing higher levels of Ang-1–7, can be a solution for COVID-19. For this purpose, plasma was obtained from plasma donors who met the conditions suitable for the scope of the study (containing high angiotensin (1−7)), and was given to patients with severe and progressive COVID-19.

Therefore, we hypothesize that the transfusion with plasma, which is obtained from these individuals (we named it “peptide plasma”), the overactive renin/Angiotensin/Angiotensin II pathway, which is partially responsible for the cytokine storm, can be stabilized by Ang-(1−7) peptide of plasma, and the cytokine storm can be slowed down for patients infected with SARS-CoV-2. Thus, we can save time for the body’s self immune system. Hence, the aim of this study is to determine whether convalescent angiotensin (1−7) peptide replacement therapy with plasma (peptide plasma) transfusion can be beneficial in the treatment of critically ill patients with severe COVID-19.

2. Material and method

2.1. Determination of the study group

This study was conducted in Health Sciences University Kanuni Sultan Suleyman Training and Research Hospital, which was designated as a pandemic hospital. The Ministry of Health and the local ethics committee approved the study (Ethics Committee approval number: KAEK/2020.05.49). The patients in the pandemic service of our hospital, whose tomography findings were compatible with bilateral diffuse pulmonary involvement of COVID-19, the clinical and/or laboratory status deteriorated with standard treatment, whose oxygen saturation fell below 94% despite the nasal oxygen support, were included. The peptide plasma transfusion was administered to those patients. Clinical and laboratory data were all recorded. Adults who were hospitalized in the pandemic ward with the diagnosis of COVID-19 included upon individual informed written consent. All participants were evaluated with nasopharyngeal swab polymerase chain reaction (PCR) and chest computed tomography (CCT). The standard treatment protocol recommended by the Ministry of Health was applied for all cases.

The study was reported according to the *Consolidated Standards of Reporting Trials* guidelines and registered on ClinicalTrials.gov (number: NCT04375124) on May 1, 2020.

2.2. Study population

Patients with laboratory confirmed COVID-19, diagnosed using quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) were eligible to receive convalescent plasma treatment if they fulfilled the following criteria: [1] had severe pneumonia with rapid progression and despite antiviral treatment; [2] Oxygen saturation of 93% or less despite nasal oxygen support of 5 liters/minute or more [3] Patients with expected rapid clinical progression, with poor prognostic parameters (lymphopenia, thrombocytopenia, C reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), D-dimer elevation).

Table 1
Characteristics of 9 cases in the study group at the time of admission.

Case number	Age (year)	Gender	Smoking	Other diseases	Symptom	Blood pressure on admission (mmHg)		PCR
1	57.4	M	+	Diabetes + Asthma + Sleep apnea	Respiratory distress	130/80	Multifocal, bilateral ground glass	negative
2	54.3	M	–	Hypertension	Respiratory distress	160/100	Multifocal, bilateral ground glass and opasity	positive
3	85.7	F	+	Hypertension	Fever, respiratory distress, muscle pain	120/80	Multifocal, bilateral ground glass and opasity	positive
4	78.2	M	–	–	Respiratory distress, muscle pain	130/80	Multifocal, bilateral ground glass	positive
5	51	F	–	Diabetes and Hypertension	Fever, respiratory distress, headache, muscle pain	130/85	Multifocal, bilateral ground glass and opasity	positive
6	75.6	F	–	–	Fever, respiratory distress, fatigue	118/68	Multifocal, bilateral ground glass and opasity	positive
7	54.4	M	+	Sleep apnea	Fever, respiratory distress, insomnia, fatigue, back pain, head ache, muscle pain	130/80	Multifocal, bilateral ground glass and opasity	positive
8	52.2	M	–	–	Respiratory distress, fatigue	145/80	Multifocal, bilateral ground glass and opasity	positive
9	36.6	M	–	–	Fever, respiratory distress, fatigue	110/60	Multifocal, bilateral ground glass and opasity	positive

Table 2
Characteristics of peptide plasma donors.

Donor number	Gender	Age	Smoking	Smoking Duration (years)	Co-Morbidities	Existence of Co-Morbidities (years)	Medications	Duration of using blood pressure medication (years)
1	M	57	0		Hypertension	8	ACE Inhibitor	8
2	M	58	1	35	COLD	8	ACE Inhibitor	7
3	F	56	0		T1DM	5	ACE Inhibitor	5
4	M	55	0		Prostate cancer	25	ACE Inhibitor	25
5	F	55	1	40	Coronary artery disease	6	ACE Inhibitor	6
6	M	50	1	10	Coronary artery disease	5	ACE Inhibitor	4

2.3. Selection of peptide plasma donor candidates

Transfusion with plasmas, with the following donor characteristics, was planned for the COVID 19 patient who deteriorated despite receiving the standard treatment specified in the national guideline:

1. Age 50 years and over (Days over 50 and not over 61 years old).
2. In case of female gender, no pregnancy history (birth/miscarriage/abortion).
3. Smoker.
4. Having a chronic disease (Hypertension, Diabetes, Heart disease, Asthma, COPD).
5. Using ACE inhibitor or ARB antihypertensive medication.
6. Not in an active period of any kind of chronic disease.

Must have at least 3 of the 6 different characteristics above:

In order to be sure that the donor candidate does not have a COVID-19 infection, the researcher will look for COVID Ig G negativity with a laboratory test.

1. Individuals who have COPD or asthma but have an attack at the time of donation.
2. Cancer patients, diabetes patients using insulin.
3. Those with organ failure (such as cirrhosis, dialysis patient).
4. People who have been infected with COVID-19.
5. People who have received blood transfusions cannot be accepted as a donor.

The order of the operations to be applied in the study:

1. Appendix 1 Inquiry Form will be fulfilled for Peptide Plasma Volunteer Donors.
2. The peptide plasma to be given to the patient must be compatible with the patient's ABO blood group (AB blood group is the general donor in plasma transfusion). Rh blood group can be ignored.
3. They will be checked for COVID-19 IgG Negativeness to make sure they don't have the disease.
4. The donor will be asked to fulfill in the Appendix2-Plasma Volunteer Donor Consent Form stating that he has donated plasma on a voluntary basis.
5. Microbiological screening tests of donor candidates (serologically HBsAg, anti-HCV, anti-HIV 1–2 and anti-syphilis Ab tests and, if possible, HBV-DNA, HCV-RNA, HIV 1,2-RNA, Nucleic tests in accordance with national legislation Acid Scan (NAT) tests) should be negative.
6. 1 ml of the volunteer's plasma will be taken by the researcher, and blood will be taken into an Eppendorf tube to determine the serum Angiotensin (1−7) level and stored at − 80 degrees.
7. Peptide plasma donation can be made up to 3 times in a month, once every 7–10 days, provided that the date of the first donation is accepted as the start date.
8. Plasma will be separated from the volunteer by apheresis.
9. Preferred delivery/transfusion timing: Plasma is given within 6 h of collection. If it cannot be given within 6 h after collection, it will be frozen and stored.

Table 3
Changes in clinical and laboratory parameters of the subjects in the study group before and after peptide plasma transfusion.

Number	Day of hospitalization	Plasma therapy	Saturation	Need for Oxygen	Blood Pressure	Lung stage	Blood Glucose Level	CRP	LDH	Ferritin	D-Dimer	Lymphocyte	Platelet count
1.	1.		93	Yes	130	2	232	13.34	329	895	1.19	1.8	180
	9.		92	Yes	110		90	136	294	1914	1.27	1.2	148
	10	Plasma	97	No	120		184	223	300	2611	1.02	0.8	188
	11	Plasma	94	No	130		193	32	274	1621	1.08	1.0	249
	12		97	No	110								
2.	1.		80	Yes	160	3	145	303	552	840	1.86	0.4	269
	2.	Plasma	90	Yes	130								
	3.	Plasma	88	Yes	162		163	103	512	707	2.6	0.2	293
	5.		93	Yes	150		137	97	496	619	3.9	0.5	250
3.	1.		97	No	130		132	89	496	600	3.8	0.7	310
	13.		95	No	110	3	99	89	245		5.16	0.7	330
	14.	Plasma	93	Yes	110								
	15.	Plasma	99	No	116		161	71	303	341	1.91	2.0	338
	16.		90	Yes	138		130	61	285	300	1.73	2.0	399
4.	1.		95	Yes	130	2							
	4.		93	Yes	124		286	110	546	1121	0.20	0.5	141
	5.	Plasma	91	Yes	110								
	6.	Plasma	96	No	138		167	33	440	1573	0.90	0.6	208
	7.		96	No	125		142	17	304	962		0.8	289
5.	1.		91	Yes	130	3	189	23	228	271	0.69	2.9	293
	4.		86	Yes	120		161	158	457	353	0.85	1.9	298
	5.	Plasma	91	Yes	100								
	6.	Plasma	93	Yes	130								
	8.		98	No	130		132	59	355	492	0.64	2.5	466
6.	1.		94	Yes	118	3							
	3.		96	Yes	76		172	191	368	306	0.31	0.6	115
	4.	Plasma	93	Yes	130								
	5.	Plasma	92	Yes	120								
	6.		96	Yes	105		142	68	377	227		1.0	210
7.	1.		90	Yes	110	3	127	153	254	454	0.96	1.0	137
	9.		87	Yes	120		117	17	240	520	2.1	1.3	347
	10	Plasma	84	Yes	130		111	7.4	168	364	1.44	1.5	298
	11	Plasma	85	Yes	110		186	6.3	250	388	1.39	1.3	250
	12		90	Yes	110		163	7.1	222	351	1.0	1.0	202
8.	1.		97	Yes	146	3	190	135	593	2993	0.2	0.6	97
	3.		89	Yes	91								
	4.	Plasma	89	Yes	130		165	108	432	3531	0.6	0.3	148
	5.	Plasma	90	Yes	120								
	6.		95	No	121		108	54	430	1707	0.7	0.5	225
	7.		94	No	109		221	45	301	870	2	0.5	232
9.	1.		94	Yes	118	3							
	7.		92	Yes	160			95	585	560	2	1.1	430
	8.	Plasma	95	Yes	134		140	42			0.8	1.3	353
	9.	Plasma	96	Yes	130								
	10.		94	Yes	130		84	13.5	530	448	1.2	1.4	693
	11		96	No	118		79	3.8	369	523	0.52	2.9	637

2.4. Peptide plasma clinical use criteria

1. Hospitalization in the COVID-19 inpatient clinic of our hospital.
2. Compatibility of CT findings with COVID-19 and presence of bilateral diffuse involvement.
3. Oxygen saturation of 93% or less despite nasal oxygen support of 5 liters/minute or more
4. Patients with expected rapid clinical progression, with poor prognostic parameters (lymphopenia, thrombocytopenia, CRP, ferritin, LDH, D-dimer elevation)
5. Progression despite standard treatment in the national guideline

2.5. Peptide plasma transfusion application

1. For each patient, 400ml/day of ABO-compatible peptid plasma was administered for 2 days (without interruption) on the same day it was obtained from the donor.
2. The patient's inflammation markers and changes in lung imaging were monitored and recorded after 48h of transfusion.
3. Since the aim is to increase the patient's serum Ang-(1−7) level, plasma infusion should not be interrupted. Therefore, infusions should be started after the adequate plasma is supplied.

2.6. Plasma peptide level measurement

To determine the peptide levels, plasma samples were extracted using a method modified from the study of Mordwinkin et al. [15] Briefly, plasma samples were extracted using Sep-Pak C18 Plus Light Cartridge (Waters Corporation MA, US). The cartridges were washed with 5 ml 96% ethanol and 15 ml dH_2O for activation. Plasma samples containing 0.5% formic acid were applied to the column. After application of the plasma samples, the columns were washed with 3 ml dH_2O and dried by passing 5 ml of air. The adsorbed peptides eluted with 5% formic acid solution 1.5x of the first plasma sample.

Then, the extracted samples were analyzed with HPLC-MS system to determine the peptide levels. Shimadzu LCMS-2010 EV model mass spectrometry and Shimadzu LC-20 modular HPLC system were used as HPLC-MS device.

In HPLC part, chromatographic separation was carried out using a Grace Kromasil 5 μm C18 column. Water (0.1% formic acid) was used as

Table 4
Peptide pre-plasma, plasma day, and post-plasma clinical and laboratory data of the cases.

	Data for days before plasma	Plasma administration day	72 h after plasma
Oxygen saturation	93 Oxygen support (min: 80- max:99)	92.5 High flow oxygen (min:84- maks: 99)	95 Room air (min:89- maks:99)
Systolic blood pressure	118 (min:76- max: 160)	130 (min:100 -max:162)	120 (min:105 -max:150)
Diastolic blood pressure	70 (min:40- max: 100)	71 (min:60- max: 97)	74 (min:54- max: 97)
Blood Glucose	119 (min:75- max: 286)	165 (min:111- max: 193)	137 (min:79- max: 221)
Urea	32.5 (min:17- max: 82)	46 (min:15- max: 80)	46 (min: 8- max:119)
Creatinine	0.74 (min:17- max: 82)	0.6 (min:0.46- max:0.97)	0.6 (min:0.43- max: 1.2)
ALT	17.5 (min:11- max: 55)	46.5 (min:34- max: 135)	40 (min:26- max: 101)
AST	23.5 (min:13- max: 46)	31.5 (min:27- max: 72)	38 (min:31- max: 104)
GGT	27.5 (min:18- max: 132)	43 (min:20- max: 80)	115 (min:19- max: 202)
ALP	74.5 (min:63- max: 105)	70 (min:61- max: 85)	94 (min:48- max: 113)
LDH	295.5 (min:203- max: 485)	262 (min:168- max: 300)	355 (min:222- max: 530)
CK	62 (min:38- max: 799)	46 (min:32- max: 63)	69 (min:35- max: 156)
Amylase	38 (min:14- max: 130)	92 (min:49- max: 137)	40 (min:34- max: 115)
Lipase	25.5 (min:4.6- max: 77)	54.8 (min:20- max: 114)	30.6 (min:19.7- max: 76)
Na	136 (min:128- max: 140)	135 (min:131- max: 138)	139 (min:134- max: 142)
K	4 (min:3- max: 5.8)	4.3 (min:4- max: 4.8)	4.3 (min:3- max: 4.8)
Cl	98 (min:90- max: 108)	93 (min:91- max: 93)	100 (min:93- max: 106)
CRP	116 (min:17- max: 355)	7.42 (min:6.3- max: 223)	57.5 (min:7.1- max: 97)
Procalcitonin	0.23 (min:0.03- max:433)	0.09 (min:0.04- max:0.40)	0.08 (min:0.05- max:11)
Ferritin	529 (min:8.3- max: 2993)	1573 (min:364- max: 3531)	578 (min:227- max: 1707)
WBC	7.5 (min:3.2 -max:52.7)	5.7 (min:3.3-max:11.2)	9 (min:5- max:14.2)
Hgb	12.1 (min:7-max:15.5)	12.1 (min:8.4-max:14.6)	13.8 (min:9.9- max:16.2)
Neutrophil	5.2 (min:2.1- max:48.6)	3.4 (min:1.3-max:8.4)	6.7 (min:3.4- max:13.1
Lymphocyte	1.2 (min:0.4-max:2.6)	1.1 (min:0.6-max:1.5)	
Monocyte	0.6 (min:0.07- max:1.8)	0.4 (min:0.37-max:0.7)	1.2 (min:0.5- max:2.9) 0.50 (min:0.3- max:0.84)

Table 5
Recovery status of the cases.

Patient Number	Hospital stay (days)	Recovery/Final status
1	13	Discharge
2	11	Discharge
3	18	Discharge
4	14	Discharge
5	12	Discharge
6	6	Exitus
7	30	Discharge
8	11	Discharge
9	13	Discharge

Table 6
Plasma Angiotensin (1–7) level measurement of peptide plasma donors.

	Plasma Angiotensin (1–7) level
Donor number	*pg/ml*
1	97
2	126
3	80
4	93
5	112
6	158

mobile phase A and acetonitrile (0.1% formic acid) was used as mobile phase B. Flow rate was 0.5 ml/min. and the column temperature was 35 °C. The gradient during separation was 5% mobile phase B in the first 5 min and 5–95% mobile phase B between 5 and 35 min. Chromatograms were acquired using UV detector operating at 210.

Total ion chromatograms and mass spectra were taken with the mass spectrometry add-on. In mass spectrometry, the detector voltage was 1.5 kV and the interface voltage was 1.5 kV. Nebulizer gas flow was 1.5 L/min and the heat block temperature was 120 °C. Positive ion mode was used to detect the peptide.

The detectors were calibrated for the peptide by loading various concentrations of the standard peptide of Ang-(1−7) (90% purity) (asp-arg-val-tyr-ile-her-pro) purchased from Genscript. The ions for the *m/z* values of 301.70 or 301.10 ($[M + 3H^+]^{3+}$), 315.40 $[M + H^+ + 2Na^+]^{3+}$, 329.30 $(M + 3H^+ + 2CH3CN]^{3+}$) ve 452.10 ($[M + 2H^+]^{2+}$) were selected from the total ion chromatograms for the quantitation of the peptide in the standard and the samples.

2.7. Statistical analysis

The quantitative data were described as the mean ± standard deviation (SD) in case of normal distribution, or as the median (min-max) otherwise. The qualitative data were described by the number of cases (proportion, %).

3. Results

Patients with bilateral diffuse pulmonary involvement, whose tomography findings were compatible with COVID-19, were followed up in the pandemic service of our hospital. In our COVID-19 cases,

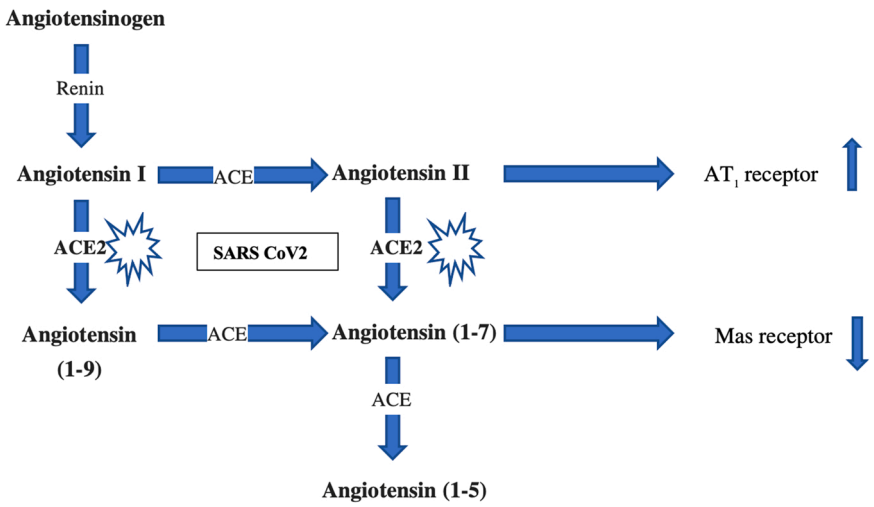

Fig. 1. Disruption of angiotensin II/Angiotensin (1−7) balance with the effect of SARS CoV2 virus.

Table 7
Possible events that may occur with disruption of Angiotensin II / Ang-(1–7) balance.

Possible event	
Cardiac fibrosis, hypertrophy, Atrial Arrhythmia	[23–26]
Increased peripheral vascular resistance	[27–29]
Disruption of baroreceptor sensitivity	[30]
Insulin resistance, dyslipidemia	[31–33]
Weakening of blood-brain barrier integrity, learning, disability, amnesia, ischaemic stroke	[34,35]
Decrease in spermatogenesis and ovulationDecreased sexual steroids synthesis	[36]
Increase in inflammation and oxidative stress, Increased tendency to thrombosis, Endotelitis	[23–26, 36]
Facilitating the formation and spread of cancer	[36]
Fibrosis and inflammation in organs such as lungs, liver and kidney.	[36–38]

favipiravir, low molecular weight heparin treatment, which is included in the treatment protocol of the ministry of health, was started. Nine patients with oxygen saturation of 93% and below despite nasal oxygen support, whose clinical and/or laboratory deteriorated, were identified. The youngest of the cases was 36 years old, and the oldest patient was 85 years old. 6 of the 9 cases had male gender. 3 cases had been smoking for more than 10 years. 4 cases had at least one chronic disease (Diabetes, Asthma, Hypertension, Sleep Apnea). Chest CT findings of the cases were evaluated in 5 stages: stage 0 is the lung being completely normal, stage 1; light one-sided ground glass image, stage 2; multifocal double-sided ground glass image, stage 3; multifocal bilateral ground glass, and stage 4; opacity, air bronchogram, bilateral ground glass and opacity, respectively. At the time of admission, the chest tomography findings of 7 cases were stage 3 and 2 patients were stage 2 (Table 1). The characteristics of the plasma donors were given in Table 2. Peptide plasma transfusion was applied to 9 patients whose clinical status and/or laboratory profile deteriorated and who needed intensive care for 2 days. Peptide plasma treatment and 72 h of follow-up were shown in Tables 3 and 4.

- Case 1 was given peptide plasma on the 10th day, while the saturation was < 93% under oxygen support from the 9th day. On the day the plasma was given, his saturation increased to 97%, eliminating the need for oxygen support. This patient was discharged on the 13th day.
- Case 2 was admitted to the hospital with 80% saturation, oxygen support was started, and he was planned to be follow-up in the intensive care unit. On the second day, peptide plasma was obtained and administered. His saturation increased to 90%. Oxygen support did not end in our 72-hour follow-up after plasma. However, the patient was discharged on the 11th day without the need for intensive care.
- Case 3 had a saturation of 93% on the 14th day of hospitalization, and peptide plasma therapy was administered when oxygen support was initiated. With the treatment, the saturation increased and the need for oxygen disappeared. However, on the 16th day, their re-saturation dropped to 90%. Oxygen support was re-started. Our patient was finally discharged on the 18th day without oxygen support.
- Case 4 is being followed up with oxygen support, and peptide plasma treatment was applied on the day when the saturation decreased to 91%. After peptide plasma treatment, the need for oxygen support disappeared. He was discharged on the 14th day of his hospitalization.
- While case 5 was receiving oxygen support, peptide plasma treatment was applied, with a saturation of 91% on the 5th day. Saturation increased with treatment. On the eighth day, the need for oxygen disappeared.
- While case 6 was being followed in oxygen support, peptide plasma therapy was applied when saturation was measured as 93% on the 4th day. However, a similar response was not obtained in this case. Oxygen requirement increased gradually and, he died on the 6th day after the intensive care process (Table 5).
- While patient number 7 was being followed up with oxygen support, peptide plasma therapy was administered when the saturation dropped below 90%. Response to treatment was obtained, saturation increased. The discharge took place on the 30th day.

Table 8
Angiotensin II/Ang-1–7 balance in different conditions.

	Chronic disease	Chronic disease and ACE inhibitor/ ARB use	COVID with chronic disease	Healthy Individuals	Smoker	Pregnancy (2–3. trimaster)	Preeclampsia
Angiotensin II	•••	••••	••••	•	••	•••	•••
Ang-1–7	•••	••••	•	•	••	•••	••

- While case 8 was being followed up with oxygen support, peptide plasma therapy was applied when the saturation fell below 90% on the 4th day. Response to treatment was obtained, saturation increased. The discharge occurred on the 11th day.
- On the 7th day of follow-up case 9, peptide plasma was administered when the saturation was 92 mmHg despite oxygen support. On the day of treatment, the saturation increased to 95 mmHg. The patient was discharged on the 13th day.

Observationally, there was a general increase in the platelet count of the cases following the peptide plasma treatment. Due to the limited number of cases, statistical significance could not be established for other laboratory data. Angiotensin (1−7) levels were determined in the plasmas used in our study. As expected, they were found to contain -30-50% higher Ang-(1−7) than normal healthy individuals (Table 6).

4. Discussion

The SARS CoV-2 virus continues to surprise the scientific world with its different faces. While the virus does not cause any symptoms or goes with mild symptoms in some individuals; for some people, it plays with the balances in the body, which can be fatal. Classical drugs and treatment methods were used against this new enemy and each country created various treatment protocols. The main approach of the struggle is to prevent the virus from multiplying and to suppress the excessive immune response created by the virus. However, an effective treatment approach has not been demonstrated yet. Since some patients worsen due to the chronic inflammatory response that continues within 3–6 months after the recovery period, and they are hospitalized in quite different clinics or die. Thus, we cannot rejoice even for individuals who have survived following the COVID-19.

Ang II is elevated in COVID-19 positive plasma [7]. When SARS-CoV-2 binds to the ACE-2 receptor, endocytosis and proteolytic cleavage is triggered [16]. This leads to a decrese in the ACE-2 activity with increased levels of Ang II together with limited production of Ang-(1−7) [16] (Fig. 1). In the lack of the counter-regulatory effect of ACE 2 activity, clinical picture of COVID-19 worsens in addition to the uncontrolled cytokine mass [17].

Male gender, advanced age (>60 years), pre-existing chronical diseases including metabolic syndrome, cardiovascular diseases and ARDS secondary to SARS-CoV-2 are reported to be associated with poor outcome of COVID-19. Not suprisingly, almost all of these poor prognostic factors are related to lower Ang-(1−7) blood levels or decreased ACE 2 activity.

Besides, given the effects of Ang-(1−7), the pathophysiology of the different clinical pictures [18–20] in the postinfectious period in adult SARS-CoV-2 patients with chronic disease may be due to the failure to restore the Ang II/Ang-(1−7) balance (Table 7). Hence, the impaired balance of Ang II / Ang-(1−7) is suggested to be causative for multiple system dysfunctions including lung, heart, kidney, and brain (Table 7). The possible balance of Angiotensin II/Angiotensin 1–7 under different conditions is shown in Table 8.

The level of Ang-(1−7) was measured 8–10 times higher in the 'peptide plasmas' of study than healthy individuals. No long COVID-19 clinic was observed in the 6-month follow-up of the discharged cases.

In a study by Schwaighofer et al. [21] they found that the level of angiotensin II increased in proportion to the severity of the COVID-19 disease: while angiotensin II level showed low values such as 16.3 pmol/L in mild covid cases, this value could reach as high as 680 pmol/L in severe covid cases. The level of Ang-(1−7) that we detected in donor plasmas that did not experience COVID-19, was 2,5–10 times higher than the severe COVID-19 cases in the study of Burden et al. In another study, the Ang-(1−7) level at admission was found to be even lower in patients who needed to be hospitalized in the intensive care unit [22]. Considering that low Ang-(1−7) levels were observed at admission, it is suggested that Ang-(1−7) supplementation early in the disease course may be a reasonable therapeutic strategy, while recombinant ACE2 supplementation alone may not be an effective strategy to overcome the Ang-(1−7) deficit in COVID-19. The physiology of RAAS is complex and it has been stated that it can be affected by many variables, including the disease itself and underlying comorbidities [22].

The ACE enzyme, which metabolizes angiotensin 1–7, is mainly located in the lung. In SARS-CoV-2 patients, Ang-(1−7) in the plasma to be given due to lung damage cannot be metabolized immediately. Therefore, we suggest that Ang-(1−7)) supplementation therapy will work against SARS-CoV-2 infectious and post-infectious period, whether in the form of plasma or synthetic peptide.

In our study, the angiotensin II/Angiotensin (1−7) ratio could not be measured due to technical impossibilities. Ang1,7 is difficult to detect because of the potential for variable sensitivity and specificity as well as rapid degradation. Hence, confirmation studies using other biochemical techniques should be performed.

Our study is a preliminary study and we suggest that it should be included in the treatment of COVID-19, in case of similar results are found in future studies with larger samples and more diverse control groups by measuring the Angiotensin II / Angiotensin (1−7) ratios in a larger number of participants.

Angiotensin 1–7 is metabolized mainly by ACE in the lungs [35]. In COVID-19 patients, angiotensin 1–7 in the plasma to be given cannot be metabolized immediately due to lung damage. Therefore, we suggest that Ang-(1−7)) supplementation therapy will work against SARS-CoV-2 infectious and post-infectious period, whether in the form of plasma or synthetic peptide. Ang (1−7) is detected in plasma right after subcutaneous application, had a half-life of 30 mins and a maximum level is measured within an hour [35]. The overactive renin / Angiotensin / Angiotensin II pathway, which is partially responsible for the cytokine storm in COVID-19, can be balanced with the angiotensin (1−7) peptide delivered by this plasma and the cytokine storm may be slowed. Ang-(1−7) in plasma to be administered in SARS-CoV-2 patients due to lung damage cannot be metabolized and eliminated immediately. Therefore, we suggest that Ang- (1−7) supplement will also be lifesaving in the prevention and/or treatment of SARS-CoV-2 postinfectious diseases.

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CRediT authorship contribution statement

Hasan Onal: Conceptualization, Writing – original draft, Methodology, Software. **Nurcan Ucuncu Ergun:** Data curation, Writing – original draft. **Bengu Arslan:** Data curation, Visualization, Investigation. **Seyma Topuz:** Supervision, Data curation. **Seda Yilmaz Semerci:** Writing – review & editing, Software, Validation. **Osman Mutluhan Ugurel:** Writing – review & editing, Validation. **Murat Topuzogullari:** Methodology, Software, Validation. **Ali Kalkan:** Data curation, Writing – original draft. **Sengul Aydin Yoldemir:** Writing – review & editing, Data curation. **Nurettin Suner:** Data curation, Writing – review & editing, Supervision **Ali Kocatas:** Writing – review & editing, Supervision.

Conflict of Interest Statement

None of the authors have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript.

References

[1] Fountain JH, Lappin SL. Physiology, renin angiotensin system. StatPearls Treasure Island (FL): StatPearls Publishing Copyright © 2021. StatPearls Publishing LLC; 2021.

[2] Grasselli G, Greco M, Zanella A, Albano G, Antonelli M, Bellani G, et al. Risk factors associated with mortality among patients with COVID-19 in intensive care units in Lombardy, Italy. JAMA Intern Med 2020;180(10):1345–55.

[3] Hanssens M, Keirse MJ, Spitz B, Van Assche FA. Measurement of individual plasma angiotensins in normal pregnancy and pregnancy-induced hypertension. J Clin Endocrinol Metab 1991;73(3):489–94.

[4] Oelkers WK. Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. Steroids 1996;61(4):166–71.

[5] Brosnihan KB, Neves LA, Anton L, Joyner J, Valdes G, Merrill DC. Enhanced expression of Ang-(1-7) during pregnancy. Braz J Med Biol Res 2004;37(8): 1255–62.

[6] Merrill DC, Karoly M, Chen K, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) in normal and preeclamptic pregnancy. Endocrine 2002;18(3):239–45.

[7] Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-converting Enzyme 2: SARS-CoV-2 receptor and regulator of the renin-angiotensin system: celebrating the 20th anniversary of the discovery of ACE2. Circ Res 2020;126(10):1456–74.

[8] Santos RAS. Angiotensin-(1-7) A Comprehensive Review / edited by Robson Augusto Souza Santos. first ed. Imprint: Springer: Cham: Springer International Publishing; 2019.

[9] Soro-Paavonen A, Gordin D, Forsblom C, Rosengard-Barlund M, Waden J, Thorn L, et al. Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. J Hypertens 2012;30(2):375–83.

[10] Luque M, Martin P, Martell N, Fernandez C, Brosnihan KB, Ferrario CM. Effects of captopril related to increased levels of prostacyclin and angiotensin-(1-7) in essential hypertension. J Hypertens 1996;14(6):799–805.

[11] Yamada K, Iyer SN, Chappell MC, Ganten D, Ferrario CM. Converting enzyme determines plasma clearance of angiotensin-(1-7). Hypertension 1998;32(3): 496–502.

[12] Shaltout HA, Westwood BM, Averill DB, Ferrario CM, Figueroa JP, Diz DI, et al. Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: evidence for ACE2-dependent processing of Angiotensin II. Am J Physiol Renal Physiol 2007;292(1):F82–91.

[13] Brake SJ, Barnsley K, Lu W, McAlinden KD, Eapen MS, Sohal SS. Smoking upregulates angiotensin-converting Enzyme-2 receptor: a potential adhesion site for novel coronavirus SARS-CoV-2 (Covid-19). J Clin Med 2020;9:3.

[14] Yilin Z, Yandong N, Faguang J. Role of angiotensin-converting enzyme (ACE) and ACE2 in a rat model of smoke inhalation induced acute respiratory distress syndrome. Burns 2015;41(7):1468–77.

[15] Mordwinkin NM, Russell JR, Burke AS, Dizerega GS, Louie SG, Rodgers KE. Toxicological and toxicokinetic analysis of angiotensin (1–7) in two species. J Pharm Sci 2012;101(1):373–80.

[16] Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005;11(8):875–9.

[17] Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004;203(2):631–7.

[18] Schwensen HF, Borreschmidt LK, Storgaard M, Redsted S, Christensen S, Madsen LB. Fatal pulmonary fibrosis: a post-COVID-19 autopsy case. J Clin Pathol 2020.

[19] Ferrario CM, Trask AJ, Jessup JA. Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. Am J Physiol Heart Circ Physiol 2005;289(6):H2281–90.

[20] Petty WJ, Miller AA, McCoy TP, Gallagher PE, Tallant EA, Torti FM. Phase I and pharmacokinetic study of angiotensin-(1-7), an endogenous antiangiogenic hormone. Clin Cancer Res 2009;15(23):7398–404.

[21] Reindl-Schwaighofer R, Hödlmoser S, Eskandary F, Poglitsch M, Bonderman D, Strassl R, et al. ACE2 elevation in severe COVID-19. Am J Respir Crit Care Med 2021;203(9):1191–6.

[22] Henry BM, Benoit JL, Berger BA, Pulvino C, Lavie CJ, Lippi G, et al. Coronavirus disease 2019 is associated with low circulating plasma levels of angiotensin 1 and angiotensin 1,7. J Med Virol 2021;93(2):678–80.

[23] Pinheiro SVB, Ferreira AJ, Kitten GT, da Silveira KD, da Silva DA, Santos SHS, et al. Genetic deletion of the angiotensin-(1-7) receptor Mas leads to glomerular hyperfiltration and microalbuminuria. Kidney Int 2009;75(11):1184–93.

[24] Santos RA, Castro CH, Gava E, Pinheiro SV, Almeida AP, Paula RD, et al. Impairment of in vitro and in vivo heart function in angiotensin-(1-7) receptor MAS knockout mice. Hypertension 2006;47(5):996–1002.

[25] Rabelo LA, Xu P, Todiras M, Sampaio WO, Buttgereit J, Bader M, et al. Ablation of angiotensin (1-7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction. J Am Soc Hypertens 2008;2(6):418–24.

[26] Peiró C, Vallejo S, Gembardt F, Azcutia V, Heringer-Walther S, Rodríguez-Mañas L, et al. Endothelial dysfunction through genetic deletion or inhibition of the G protein-coupled receptor Mas: a new target to improve endothelial function. J Hypertens 2007;25(12):2421–5.

[27] Santos RA, Ferreira AJ, Verano-Braga T, Bader M. Angiotensin-converting enzyme 2, angiotensin-(1-7) and Mas: new players of the renin-angiotensin system. J Endocrinol 2013;216(2):R1–r17.

[28] Iusuf D, Henning RH, van Gilst WH, Roks AJ. Angiotensin-(1-7): pharmacological properties and pharmacotherapeutic perspectives. Eur J Pharmacol 2008;585 (2–3):303–12.

[29] Zimmerman D, Burns KD. Angiotensin-(1-7) in kidney disease: a review of the controversies. Clin Sci (Lond) 2012;123(6):333–46.

[30] Guimaraes PS, Oliveira MF, Braga JF, Nadu AP, Schreihofer A, Santos RA, et al. Increasing angiotensin-(1-7) levels in the brain attenuates metabolic syndrome-related risks in fructose-fed rats. Hypertension 2014;63(5):1078–85.

[31] Giani JF, Mayer MA, Muñoz MC, Silberman EA, Höcht C, Taira CA, et al. Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. Am J Physiol Endocrinol Metab 2009;296(2): E262–71.

[32] Echeverría-Rodríguez O, Del Valle-Mondragón L, Hong E. Angiotensin 1-7 improves insulin sensitivity by increasing skeletal muscle glucose uptake in vivo. Peptides 2014;51:26–30.

[33] Santos SH, Braga JF, Mario EG, Pôrto LC, Rodrigues-Machado Mda G, Murari A, et al. Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). Arterioscler Thromb Vasc Biol 2010;30(5):953–61.

[34] Wu J, Zhao D, Wu S, Wang D. Ang-(1-7) exerts protective role in blood-brain barrier damage by the balance of TIMP-1/MMP-9. Eur J Pharmacol 2015;748: 30–6.

[35] Allred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1—7) metabolism in pulmonary and renal tissues. Am J Physiol Renal Physiol 2000;279 (5):F841–50.

[36] Passos-Silva DG, Verano-Braga T, Santos RA. Angiotensin-(1-7): beyond the cardio-renal actions. Clin Sci ((Lond)) 2013;124(7):443–56.

[37] Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Crit Care 2017;21(1):234.

[38] Haschke M, Schuster M, Poglitsch M, Loibner H, Salzberg M, Bruggisser M, et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. Clin Pharmacokinet 2013;52(9): 783–92.