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

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ARTICLE INFO

Keywords:
COVID-19
ACE2
Angiotensin (1–7)
SARS-CoV-2
Plasma

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.
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 I (ATI) / Angiotensin II pathway (inflammation producing pathway) via the ATI receptor, angiotensin 1 and 2 are degraded by the angiotensin-converting enzyme 2 (ACE2) / Angiotensin (1–7) pathway (anti-inflammatory) and Angiotensin (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 pregnant [5,6]. ACE inhibitors (ACE-I) prolong 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 angiotensin 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).
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.
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 peptide 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$_2$O 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$_2$O 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 mobile phase. To determine the peptide levels, plasma samples were extracted using a method modified from the study of Mordwinkin et al. [15].

| 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 count | Platelet count |
| 1. | 1. | 93 | Yes | 130 | 2 | 232 | 13.34 | 329 | 895 | 1.19 | 1.8 | 180 |
| 9. | 2. | 92 | Yes | 110 | 1 | 90 | 136 | 294 | 1914 | 1.27 | 1.2 | 148 |
| 10. | Plasma | 97 | No | 120 | 2 | 184 | 223 | 300 | 2611 | 1.02 | 0.8 | 188 |
| 11. | Plasma | 94 | No | 130 | 1 | 193 | 32 | 274 | 1621 | 1.08 | 1.0 | 249 |
| 12. | Plasma | 97 | No | 110 | 2 | 145 | 303 | 552 | 840 | 1.86 | 0.4 | 269 |
| 2. | 1. | 80 | Yes | 160 | 3 | 145 | 303 | 552 | 840 | 1.86 | 0.4 | 269 |
| 2. | Plasma | 90 | Yes | 130 | 4 | 163 | 103 | 512 | 707 | 2.6 | 0.2 | 293 |
| 3. | Plasma | 88 | Yes | 162 | 5 | 137 | 97 | 496 | 619 | 3.9 | 0.5 | 250 |
| 5. | Plasma | 93 | Yes | 150 | 3 | 137 | 97 | 496 | 619 | 3.9 | 0.5 | 250 |
| 3. | 1. | 97 | No | 130 | 3 | 132 | 89 | 496 | 600 | 3.8 | 0.7 | 310 |
| 5. | Plasma | 95 | No | 110 | 3 | 99 | 89 | 496 | 600 | 3.8 | 0.7 | 310 |
| 13. | Plasma | 93 | Yes | 100 | 6. | Plasma | 93 | Yes | 130 | 8. | 96 | No | 130 | 2 |
| 4. | 1. | 95 | Yes | 130 | 2 | 286 | 110 | 546 | 1121 | 0.20 | 0.5 | 141 |
| 4. | Plasma | 93 | Yes | 124 | 5. | Plasma | 91 | Yes | 110 | 7. | Plasma | 96 | No | 138 |
| 6. | Plasma | 96 | No | 125 | 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. | Plasma | 91 | Yes | 100 | 5. | Plasma | 91 | Yes | 100 | 6. | Plasma | 93 | Yes | 130 |
| 8. | Plasma | 98 | No | 130 | 8. | Plasma | 98 | No | 130 | 6. | Plasma | 93 | Yes | 130 |
| 3. | 1. | 96 | Yes | 118 | 3 | 172 | 191 | 368 | 306 | 0.31 | 0.6 | 115 |
| 4. | Plasma | 96 | Yes | 76 | 5. | Plasma | 92 | Yes | 120 | 6. | Plasma | 96 | Yes | 105 |
| 5. | Plasma | 92 | Yes | 120 | 6. | Plasma | 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. | Plasma | 87 | Yes | 120 | 10. | Plasma | 84 | Yes | 130 | 11. | Plasma | 85 | Yes | 110 |
| 9. | Plasma | 87 | Yes | 120 | 11. | Plasma | 85 | Yes | 110 | 12. | Plasma | 90 | Yes | 110 |
| 7. | 1. | 97 | Yes | 146 | 3 | 190 | 135 | 593 | 2993 | 0.2 | 0.6 | 97 |
| 3. | Plasma | 89 | Yes | 91 | 4. | Plasma | 89 | Yes | 130 | 5. | Plasma | 90 | Yes | 120 |
| 5. | Plasma | 90 | Yes | 120 | 6. | Plasma | 95 | No | 121 | 108 | 54 | 430 | 1707 | 0.7 | 0.5 | 225 |
| 7. | Plasma | 94 | No | 109 | 7. | 94 | No | 109 | 221 | 45 | 301 | 870 | 2.0 | 0.5 | 232 |
| 9. | 1. | 94 | Yes | 118 | 3 | 140 | 42 | 250 | 388 | 1.39 | 1.3 | 250 |
| 7. | Plasma | 92 | Yes | 160 | 8. | Plasma | 95 | Yes | 134 | 10. | Plasma | 94 | Yes | 130 |
| 9. | Plasma | 96 | Yes | 130 | 11. | Plasma | 96 | Yes | 118 | 9. | Plasma | 91 | Yes | 110 |
| 10. | Plasma | 94 | Yes | 130 | 11. | Plasma | 94 | Yes | 130 | 79 | 3.8 | 369 | 523 | 0.52 | 2.9 | 637 |

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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       | Oxygen support (min: 80- max:99) | High flow oxygen (min:94- max: 99) | Room air (min:99- max: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)       | 40 (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)       | 40 (min:35- max: 156) |
| Amylase                 | 38 (min:14- max: 130)       | 92 (min:49- max: 137)      | 92 (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: 4.6) |
| CI                      | 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:0.43)   | 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:5.3-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)      | 1.1 (min:0.6-max:1.5) |

Table 4 (continued)

|                         | Data for days before plasma | Plasma administration day | 72 h after plasma |
|-------------------------|----------------------------|---------------------------|------------------|
| 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) |

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.

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

mobile phase A and acetoniitrile (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) (aspar-arg-val-tyr-ile-her-pro) purchased from Genscript. The ions for 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,
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 91%. 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. Saturaton 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 7
Possible events that may occur with disruption of Angiotensin II / Ang-(1–7) balance.

| Possible event                                                                 | Reference |
|--------------------------------------------------------------------------------|-----------|
| Cardiac fibrosis, hypertrophy, 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 ovulation Decreased 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]   |

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. trimester) | 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 decrease 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 chronic 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 surprisingly, 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. 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.

Financial support

Our project application titled “Angiotensin (1–7) peptide replacement therapy with plasma in Covid19” numbered 8973, which we made to the 2020-IG-02 Applied Project Cooperation Call in the Field of Pharmaceutical Development opened by the Presidency of Turkish Health Institutes Strategic R&D project as supported.

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 Ugur: 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.
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