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Efficacy and safety of convalescent plasma therapy in severe COVID-19 patients with acute respiratory distress syndrome☆

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ABSTRACT

Since SARS-CoV-2 infection is rapidly spreading all around the world, affecting many people and exhausting health care resources, therapeutic options must be quickly investigated in order to develop a safe and effective treatment. The present study was designed to evaluate the safety and efficacy of convalescent plasma (CP) for treating severe cases of COVID-19 who developed acute respiratory distress syndrome (ARDS). Among 64 confirmed cases of severe COVID-19 with ARDS in this study, 32 patients received CP besides first line treatment. Their clinical response and outcome in regard to disease severity and mortality rate were evaluated and compared with the other 32 patients in the control group who were historically matched while randomly chosen from previous patients with the same conditions except for receiving CP therapy. Analysis of the data was performed using SPSS software. Patients with plasma therapy showed improvements in their clinical outcomes including a reduction in disease severity in terms of SOFA and APACHE II scores, the length of ICU stay, need for noninvasive ventilation and intubation and also showed an increase in oxygenation. They also showed reduction in mortality which was statistically significant in less severe cases with mild or moderate ARDS. Early administration of the convalescent plasma could successfully contribute to the treatment of severe COVID-19 patients with mild or moderate ARDS at risk of progressing to critical state.

☆ Trial Registration: Iranian Registry of Clinical Trials; URL: https://www.irct.ir/trial/47058; Clinical Trial Registration Number: IRCT20200409047007N1.

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1. Introduction

From 1940 to 2001, an average of 5.3 viruses have emerged per year, about 60–70% of which have been human pathogens [1]. Two outbreaks of coronaviruses in humans have occurred since 2002; SARS-CoV (2002) causing Severe Acute Respiratory Syndrome (SARS) and MERS-CoV (2012) causing Middle East Respiratory Syndrome (MERS) [2–4]. Since December 2019, the third outbreak of a rapidly transmitted coronavirus (median Ro ≈5.7) [5] named as SARS-CoV-2 has emerged, causing novel coronavirus pneumonia (respiratory disease) (NCP) later named as Coronavirus Disease 2019 (COVID-19) by WHO. Despite the early reports, recent studies calculated its reproductive number (Ro) to be 5.7 and the virus doubling time is 2.3–3.3 days indicating its high contagiousness which justifies its rapid spread throughout the world and causing a pandemic. While most COVID-19 patients experience a mild clinical course, severe cases are at high risk of rapidly developing acute respiratory distress syndrome (ARDS) particularly within 7–14 days from onset of symptoms [6] which often leads to acute respiratory failure with high mortality rates in a short time (20 times higher than that of non-severe COVID-19 patients) [7,8].

Healthcare systems are struggling to cope with the increasingly growing number of COVID-19 patients presenting to hospitals all around the world as the disease spreads rapidly [9]. Managing COVID-19 has been mostly supportive to date, such as providing supplemental oxygen to cases with mild disease and using extracorporeal membrane oxygenation for critically ill patients [10]. However, in fast evolving pandemics with no observable natural immunity in the population, effective treatment options must be quickly investigated and become available to alleviate the symptoms and reduce the mortality. Unfortunately, no specific treatment or vaccines have been proven to be effective for SARS-CoV-2 infection so far which makes it even more difficult for the pandemic to be contained. The efficacy of some specific drugs including anti-viral and anti-HIV agents (remdesivir, favipiravir [11] and Lopinavir /Ritonavir [12]) and other medications and therapeutic strategies for COVID-19 such as antimalarial drugs (chloroquine and hydroxychloroquine), a combination of hydroxychloroquine and azithromycin [13] and lopinavir + ritonavir + interferon-beta are still under investigation [9,12,14,15]. While some of these medications have been shown to be beneficial, there is still not enough clinical evidence to prove their safety and efficacy. Therefore, the treatment remains challenging. Considering the absence of specific efficacy-proven preventative and therapeutic options, attention has also been given to classical and empirical interventions as an option to treat COVID-19 patients [16,17]. Accordingly, researchers and physicians have resorted to use the plasma containing SARS-CoV-2 specific antibodies from convalescent donors to treat severe cases of COVID-19. In the late 19th century, antibody transfer was first used to fight against bacterial toxins before the discovery of antimicrobials, however, it has been applied to confer passive immunity against many other infectious diseases associated with different microorganisms since then when there was no therapeutic agent available, high risk of infection or not enough time for the body to acquire active immunity due to rapid progression of the disease [18–21].

Using convalescent plasma (CP) transfusion has also been recommended as an emergency intervention during the Spanish flu, H5N1 avian influenza, West Nile virus, SARS, MERS and Ebola outbreak which was shown to be beneficial in many cases by reducing the hospital stay and mortality rate [22–31]. Accordingly, a recent systematic review of 40 studies on CP treatment for infectious diseases including SARS, MERS, Ebola, H1N1, H5N1 and H7N9 also concluded that CP treatment could result in promoting antibody production while shortening the disease course, reducing viral load and mortality with low risk of adverse effects, suggesting that it could be potentially effective for treating COVID-19 patients [32].

Considering the value of time in the current pandemic, it is of great importance to promptly address the urgent question raised regarding CP efficacy in managing Sars-CoV-2 infection. Therefore, the present study was designed to investigate the efficacy of using convalescent plasma therapy to treat severe cases of COVID-19 suffering from ARDS.

2. Materials and methods

2.1. Study design

This clinical trial was carried out in Imam Reza hospital, the referral center for COVID-19 patients in Mashhad, Iran, from Apr 21 to May 31, 2020 and the final date of follow up was June 27, 2020 in which 64 COVID-19 inpatients suffering from ARDS were included among whom 32 patients received convalescent plasma therapy besides the regular treatment for COVID-19 patients and their clinical outcomes were compared with 32 patients in the control group with the same conditions who only received the regular treatment and no CP. To evaluate the outcomes of the intervention and compare with the control group, the patients were followed up until 4 weeks. The study protocol and design were carefully reviewed and approved by the ethics committee of Mashhad University of Medical Sciences (Ethics Committee Code: IR.MUMS.REC.1399.055, Clinical Trial Registration Number: IRCT20200409047007N1). All the participants in the study including patients (patients themselves or their guardians in case of the patient's unconsciousness) and donors signed the written informed consent.

2.2. Patients

A total number of 64 laboratory confirmed COVID-19 patients were included in the study whom met all of the Inclusion criteria which come as follows:

1. Age from 18 to 75 years
2. Developing ARDS resulted from COVID-19
3. PaO2/FiO2 $\leq$ 250 despite receiving first line treatment (hydroxychloroquin, corticosteroid and broad-spectrum antibiotics)
4. Normal Immunoglobulin A (IgA) level
5. Absence of uncontrolled Hypertension (HTN)
6. Absence of background diseases such as heart failure, chronic liver disease and Chronic obstructive pulmonary disease (COPD)
7. Systolic blood pressure $\geq$ 90 mmHg on the admission day.
8. Not being intubated
9. Glasgow Coma Scale (GCS) $\geq$ 12
10. Glomerular filtration rate (GFR) $\geq$ 30

Patients were excluded from the study if they did not consent to participate in the trial or were allergic to the plasma product.

The control group were historically selected from the previous patients using the registry system which keeps record of the detailed clinical information and outcomes of the COVID-19 patients. In order to have a better comparison, they were matched with the patients in the intervention group considering factors such as disease severity (in terms of PaO2/FiO2 (±30) on the similar day of hospitalization), age (±10 years), gender, first line treatment, background diseases (Hypertension (HTN) and Diabetes Mellitus (DM) only) and symptom day. One hundred and eleven patients were randomly chosen by a blinded technician (totally unaware of the study design, purpose and possible results), using the advanced search tool in the registry system through which the first search results appeared according to the defined criteria applied on search filters were chosen. Propensity score (PS) was calculated regarding the abovementioned variables to remove the chronology bias and control the confounding factors. Ultimately, 32 patients who were more closely matched with the patients in the intervention group, were assigned to the control group.

The day on which the convalescent plasma was transfused to the patients of the intervention group was defined as the day zero (day_0) and the following days were numbered accordingly.

2.3. Donors

Recovered COVID-19 patients between 18 and 60 years old, with initial positive reverse transcriptase–polymerase chain reaction (RT-PCR) test for SARS-CoV-2 and absence of the symptoms of the disease for at least 14 days, who were eligible to donate blood considering the Blood Donor Organization standards (except for recent SARS-CoV-2 infection) were invited to participate in the study. The final donors participating in the study included asymptomatic men who tested negative for HBS Ag, HCV Ab, HIV Ab, HTLV1 Ab, VDRL and SARS-CoV-2 nucleic acid while positive for SARS-CoV-2 IgG and IgM at the time of donating blood. Other tests including CBC, CRP, blood group and Rh factor blood test were also carried out. Plasma contents of the donors were also subjected of quality control regarding the blood cells count in order to make sure that the number of red blood cells (RBC), white blood cells (WBC) and platelets were below 0.2×10^9, 0.1×10^9 and 10×10^9 per liter, respectively. Moreover, plasma culture was performed for each donor to make sure there was no infection.

2.4. Real-Time reverse transcription polymerase chain reaction

The specimens including nasopharyngeal swabs and oropharyngeal swabs were taken and immediately placed in the tubes containing viral transport media. All sample processing and reactions were carried out under class II biological safety cabinets. Detection of SARS-CoV-2 nucleic acid was performed through real-time RT-PCR assays using commercial detection kit (*DAAN Gene, China*). Two independent primers and probes matching the open reading frame1ab (ORF1ab) and the nucleocapsid protein (N) fragments were used. RNase P gene was used as an internal control. Real-time RT-PCR was performed following the manufacturer's recommendations. Each amplicon provided a cycle threshold value (Ct value), which is the number of cycles required for the fluorescent signal. Ct values less than 40 were considered as positive results, while Ct values exceeding 40 were defined as negative results.

2.5. Enzyme-linked immunosorbent assay (ELISA)

Detection of anti-SARS-CoV-2 antibodies was carried out through two separate ELISA kits with similar procedures, each designed specifically to reveal anti-SARS-CoV-2 IgG and IgM using the plates coated by N proteins of SARS-CoV-2 (Pishtaz Teb, Iran). In each assay, diluted serum samples (1:101 for detection of IgG and 1;51 for detection of IgM) and controls were added to the plates. After a 30 min incubation at 37 °C, plates were washed and horseradish peroxidase (HRP)- labeled antigens were added. Another 30 min incubation at 37 °C was applied before the plates were washed again so that the unbound components would be eliminated. Chromogen substrate was added afterward, which was followed by adding the stop buffer in 15 min. Absorbance was measured at 450 nm and 630 nm dual-wavelength using a microplate reader. The cutoff optical density (OD) was calculated as the mean OD of negative controls plus 0.25 in case of IgM and 0.15 in case of IgG. Results were reported as an index, calculated as the OD value of the samples divided by the cutoff OD value. The cutoff value recommended by the kit manufacturer was used according to which ratios higher than 1.1 were considered positive. However, to ensure providing recipients with higher levels of antibodies, the donors included in the study were chosen among those with highest cutoff index (between 10 and 32).

2.6. Convalescent plasma preparation, therapeutic safety and transfusion

Convalescent plasma was obtained from donors by apheresis and each patient received one cycle of 600 ml fresh ABO-identical and RhD-compatible convalescent plasma in the same day and as soon as possible (within 4 h). Transfusion was administered slowly with continuous monitoring of the patients' vital signs by the clinician in order to prevent adverse outcomes associated with plasma transfusion which mainly include volume overload and allergic response to plasma contents.

In case of presenting volume overload signs, the transfusion would be paused and resumed slowly while reducing maintenance serum volume after patient's stability and administering diuretic and venous nitroglycerin. In case of mild allergic reactions such as rash, itching and mild fever to the plasma contents, the transfusion would be paused and resumed slowly after administering chlorphenamine and Corticosteroid. In case of severe hypersensitivity reactions such as hypotension and decreased oxygen saturation, the transfusion would be stopped and the complications would be controlled by chlorphenamine, epinephrine and Corticosteroid. No more plasma transfusion would be performed in such cases and the patients would be excluded from the study if they did not have two successful infusions before this one.

2.7. Monitoring patients and outcome evaluation

Patients' conditions, clinical examinations and outcomes were precisely monitored in the intervention group and were compared with the control groups. Factors considered in this regard to evaluate and compare the outcome of the intervention included length of hospitalization, need for mechanical ventilation, disease severity based on PaO2/FiO2, Sequential Organ Failure Assessment (SOFA) score (ranging from 0 to 24 with 24 indicating the highest level of severity) and APACHE II (Acute Physiology And Chronic Health Evaluation II) score (ranging from 0 to 71 where higher scores correspond to more severity) on day zero, three and the day of discharge (last day of hospitalization leading to discharge or death) and finally the mortality rate in 4 weeks (day 28) as the main outcome.

2.8. Data analysis

Analyzes were carried out using SPSS software (Version 22.0, SPSS, Inc., Chicago, IL, USA Quantitative variables were presented as mean and standard deviation while the qualitative variables were reported as frequency and percent. Comparison analysis between the two groups was performed using T-Test or Mann-Whitney U Test based on the variables' distribution. To compare the difference between the qualitative variables of the two groups, Pearson Chi-Square Test or Fisher's Exact Test were performed. Correlation analyzes were done using Spearman Correlation test. Differences were considered statistically significant where P values were less than 0.05. To assess the strength and direction of association between variables, Spearman Correlation Coefficient (r) was considered.

3. Results

Patients were matched regarding age, gender, background disease and PaO_2/FiO_2 level on the similar day (the day of plasma transfusion, referred to as day zero) in both plasma and control groups, accordingly there were no significant differences between two groups regarding these variables. Patients receiving convalescent plasma did not present any immediate and noticeable adverse effect. Table 1 Shows the demographic data and PaO_2/FiO_2 of the patients of the study on the day of plasma transfusion (Day 0) and on the same day from admission in the control group. Blood oxygen saturation (SpO2) was assessed to be $\leq$ 85% in all the patients without supplemental oxygen on the same day. Each group included 32 patients; 18 men (56.2%) and 14 women (43.8%), 13 (40.6%) and 11 (34.4%) patients had DM and HTN, respectively, among which 9 (28.1%) patients had both diseases at the same time. Mean age was calculated to be 56.69 ± 14.32 years old (58.74 ± 14.67 in the plasma group and 55.53 ± 14.10 in the control group). Patients were also classified as mild, moderate and severe (ARDS) with regard to their PaO_2/FiO_2 on day zero. Patients with PaO_2/FiO_2 in the range of 200–250, 100–200 and lower than 100 mmHg were classified as mild, moderate and severe, respectively. In total, 4, 9 and 19 patients had severe, moderate and mild ARDS in each group,

Table 1
Patients' demographic data and PaO_2/FiO_2 level on day zero.

Plasma Group	Age	Gender	$(P/F)_0$ (mmHg)	Comorbidity	Control Group	Age	Gender	$(P/F)_0$ (mmHg)	Comorbidity
1P	74	F	138	2	1C	69	F	130	2
2P	46	M	190	0	2C	46	M	172	0
3P	50	M	108	0	3C	47	M	102	0
4P	60	F	73	0	4C	68	F	92	0
5P	66	M	62	0	5C	66	M	76	0
6P	75	M	219	0	6C	71	M	230	0
7P	62	F	223	2	7C	63	F	209	2
8P	42	F	44	0	8C	34	F	68	0
9P	36	F	64	0	9C	36	F	89	0
10P	70	M	195	2	10C	71	M	199	2
11P	64	F	228	2	11C	65	F	228	2
12P	75	F	238	HTN	12C	31	F	228	HTN
13P	36	M	238	0	13C	38	M	228	0
14P	55	F	238	0	14C	50	F	238	0
15P	68	M	228	DM	15C	69	M	228	DM
16P	67	M	219	0	16C	57	M	228	0
17P	70	M	209	2	17C	74	M	201	2
18P	64	F	238	DM	18C	56	F	238	DM
19P	53	F	219	0	19C	56	F	215	0
20P	47	M	138	2	20C	47	M	155	2
21P	67	F	228	0	21C	67	F	205	0
22P	62	M	171	0	22C	58	M	175	0
23P	42	M	161	0	23C	42	M	164	0
24P	37	M	233	DM	24C	37	M	238	DM
25P	33	M	219	0	25C	41	M	238	0
26P	73	F	223	2	26C	67	F	228	2
27P	21	M	199	0	27C	24	M	190	0
28P	61	M	223	HTN	28C	67	M	238	HTN
29P	65	F	219	2	29C	64	F	219	2
30P	74	M	223	2	30C	64	M	228	2
31P	61	M	223	DM	31C	61	M	238	DM
32P	75	F	190	0	32C	71	F	185	0

$(P/F)_0$: PaO_2/FiO_2 on day zero. 1P, 2P, 3p, …, n P: number dedicated to the patients in the plasma group. 1C, 2C, 3C, …, n C: number dedicated to the matched patients in the control group (the patient with the same characteristics as the patient in the intervention group). F: female. M: Male. 0: No background disease. DM: Diabetes Mellitus. HTN: Hypertension. 2: Having both DM and HTN.

respectively.

Noninvasive ventilation (NIV) and intubation were used for19 (12 men, 7 women) and 14 cases (8 men, 6 women) in the control group, while for only 14 (9 men, 5 women) and 8 cases (4 men, 4 women) in plasma group, respectively. Although the need for NIV and intubation was less in the plasma group, no statistically significant difference was observed between the two groups (Table2).

In general, 43 patients (67.2%) including 25 in the plasma group and 18 in the control group were discharged and 21 patients (32.8%) including 7 in the plasma group and 14 in the control group died. While the number of recovered patients were higher in the plasma group in comparison with the control group, there was no statistically significant difference between the two groups (P value: 0.062 by Pearson Chi-Square Test). Moreover, there was no statistically significant difference between men and women in the need for NIV and intubation nor in the main outcome (discharge or death in 28 days). Also no significant differences were observed between patients with or without comorbidities and the abovementioned variables. Propensity score calculations showed a p value of ≤ 0.001 (Odds Ratio (OR): 1.30, with 95% Confidence Interval (CI) from 1.13 to 1.49) indicating that the odds of death in control group was 30% higher compared to the plasma group.

Table 2
Patients' outcomes in the plasma and control groups.

Variable		Plasma Group	Control Group	P value *
NIV[a]	Yes	14 (43.8%)	19 (59.4%)	0.211
	No	18 (56.3%)	13 (40.6%)	
Intubation	Yes	8 (25%)	14 (43.8%)	0.114
	No	24 (75%)	18 (56.2%)	
Main Outcome (Mortality)[b]	Death	7 (21.9%)	14 (43.8%)	0.062
	Discharge	25 (78.1%)	18 (56.3%)	

[a] Noninvasive ventilation.
[b] Main outcome (death/discharge) in 28 days.
* P values calculated by Pearson Chi-Square Test.

According to the results shown in the table 3, patients classified as mild ARDS ($200 < PaO_2/FiO_2 \leq 250$) were shown to be recovering more in the plasma group (19 out of 19 patients: 100%) compared with the control group (14 out of 19 patients: 73.7%), the difference was statistically significant (P value: 0.046 by Fisher's Exact test). While the number of discharged patients with a moderate ARDS ($100 \leq PaO_2/FiO_2 \leq 200$) was also higher in the plasma group (5 out of 9 patients: 55.6%) compared with the control group (3 out of 9 patients: 33.3%), it was not significantly different. Equal number of patients with severe ARDS (PaO_2/FiO_2 below 100) were discharged in both groups (1 patient out of 4: 25%).

Serum antibody levels (IgM and IgG) on day zero the, and the level of CRP and interleukin 6 assessed on day zero and three in the plasma group are shown in the table 4. There were no significant differences between CRP and interleukin 6 levels on day zero and their levels on the day three. There was a significant relationship between higher levels of CRP on the day three and the need for intubation and also the death outcome (P values: 0.024 and 0.028 by Mann-Whitney *U* test, respectively), however; other factors did not have any relationship with the need for intubation and mortality. None of the abovementioned factors were statistically related with the need for NIV either. Table 4 also shows the quantity of the variables associated with the length of the disease and treatment of the patients in the plasma group including the number of days from patients' symptoms to their admission to the hospital, admission to the day of plasma transfusion and symptoms to the plasma transfusion day. The mean interval time from admission to receiving convalescent plasma by patients was assessed to be 4.41 ± 2.28 days ranging from 3 to 11 days.

Table 3
Patients' main outcome according to ARDS severity.

ARDS Severity	Plasma Group Total	Discharge	Death	Control Group Total	Discharge	Death	P value*
Severe (PaO_2/FiO_2 ⟨100)	4	1 (25%)	3 (75%)	4	1 (25%)	3 (75%)	> 0.99
Moderate (100 ≤ PaO_2/FiO_2 ≤ 200)	9	5 (55.6%)	4 (44.4%)	9	3 (33.3%)	6 (66.7%)	0.637
Mild (200 < PaO_2/FiO_2 ≤ 250)	19	19 (100%)	0 (0%)	19	14 (73.7%)	5 (26.3%)	0.046

* P values calculated by Fisher's Exact Test.

Table 4
Factors associated with the inflammation, immune response, length of the disease and treatment in patients in the plasma group.

Variables	Mean ± Standard Deviation	Range
Symptoms to Admission (day)	6.03 ± 2.79	2–11
Admission to Plasma (day)	4.41 ± 2.28	3–11
Symptoms to Plasma (day)	10.44 ± 2.95	5–14
IgM_0	5.87 ± 5.95	0–23.52
IgG_0	11.55 ± 9.48	0.01–23.74
CRP_0	130.31 ± 109.36	3.90–398
CRP_3	97.23 ± 102.50	0.40–355.80
Interleukin 6_0	118.79 ± 69.74	10–299
Interleukin 6_3	147.24 ± 163.04	8.90–700

IgM_0: Serum IgM level on day zero, IgG_0: Serum IgG level on day zero, CRP_0: CRP on day zero, CRP_3: CRP on the day Three, Interleukin 6_0: Interleukin 6 on day zero, Interleukin 6_3: Interleukin 6 on the day three.

Table 5
Comparison of the quantitative variables associated with the disease severity in the two groups.

Variable	Group	Mean ± Standard Deviation	Range	P value *
Admission to Discharge (day)	Plasma	13.91 ± 8.43	5–51	0.732
	Control	15.34 ± 10.11	5–56	
	Total	14.63 ± 9.27	5–56	
$(P/F)_0$(mm/Hg)	Plasma	188.16 ± 58.48	44–238	0.666
	Control	190.63 ± 53.66	68–238	
	Total	189.39 ± 55.69	44–238	
$(P/F)_3$(mm/Hg)	Plasma	181.28 ± 64.33	46–309	0.350
	Control	193.28 ± 58.83	72–258	
	Total	187.28 ± 61.45	46–309	
$(P/F)_d$(mm/Hg)	Plasma	275.03 ± 142.54	32–547	0.034
	Control	213.41 ± 92.76	66–331	
	Total	244.22 ± 123.26	32–547	
$SOFA_0$	Plasma	3.25 ± 1.08	2–6	0.316
	Control	3.22 ± 1.76	1–8	
	Total	3.23 ± 1.44	1–8	
$SOFA_3$	Plasma	3.47 ± 2.03	1–10	0.761
	Control	3.59 ± 1.10	2–9	
	Total	3.53 ± 2	1–10	
$SOFA_d$	Plasma	3.34 ± 3.70	0–14	0.058
	Control	3.91 ± 2.79	1–10	
	Total	3.63 ± 3.26	0–14	
APACHE II_0	Plasma	8 ± 2.60	3–13	0.995
	Control	8.28 ± 2.98	4–14	
	Total	8.14 ± 2.78	3–16	
APACHE II_3	Plasma	6.53 ± 2.34	1–12	0.001
	Control	9.69 ± 4.28	3–23	
	Total	8.11 ± 3.78	1–23	
APACHE II_d	Plasma	6.69 ± 4.17	0–17	0.023
	Control	9.25 ± 5.14	3–25	
	Total	7.97 ± 4.82	0–25	

$(P/F)_0$: PaO_2/FiO_2 on day zero, $(P/F)_3$: PaO_2/FiO_2 on the day three. $(P/F)_0$: PaO_2/FiO_2 on the last day of discharge (Last day of hospitalization: Discharge/Death). $SOFA_0$: SOFA score on day zero, SOFA3: SOFA score on the day three. $SOFA_d$: SOFA score on day of discharge. APACHE II_0: APACHE II score on day zero, APACHE II_3: APACHE II score on the day three, APACHE II_d: APACHE II score on day of discharge.

* P values calculated by Mann-Whitney U Test.

Table 5 shows the mean, standard deviation, minimum and maximum of the quantitative variables associated with the disease severity in total and also in each group. According to the table, the length of hospitalization was more in the control group. The level of PaO_2/FiO_2 on day of discharge was significantly higher in the plasma group (275.03) compared with the PaO_2/FiO_2 level in control group (213.41) (P value:0.034 by Mann-Whitney *U* Test). SOFA scores were lower in plasma group both on the day three and the day of discharge compared with the scores of the control group. However, none of the abovementioned variables showed statistically significant difference between the two groups. APACHE II scores in the plasma group decreased over time and were estimated to be less than the scores in the control group on both days (day three and the day of discharge) which were significantly different (P values: 0.001 and 0.023 by Mann-Whitney *U* Test, respectively). No significant differences were observed between any of the abovementioned variables and gender neither with having diabetes mellitus in the patients in any of the two groups, however; having hypertension was shown to be statistically correlated with higher SOFA and APACHE II scores on day of discharge in the control group (P values: 0.034 and 0.040, respectively, calculated by Mann-Whitney *U* test).

Spearman correlation analyses between different variables including IgM, IgG, CRP and Interleukin 6 on day zero and three, admission to discharge interval, PaO_2/FiO_2 level, SOFA and APACHE II scores on day zero, three and day of discharge in the plasma group showed that there were statistically significant correlation between APACHE II on day of discharge and CRP on the day three (P value: 0.025, r: 0.456), an inverse correlation between CRP on day zero and PaO_2/FiO_2 level on day of discharge (P value:0.033, r: −0.405) and also a stronger correlation (r: 0.607) between interleukin 6 on the day 3 and APACHE II score on the same day (P value: 0.013).

4. Discussion

The present study evaluated the safety and effectiveness of convalescent plasma therapy for severe COVID-19 patients with ARDS. The outcomes were compared with patients in the control group who shared the same characteristics and treatment protocol with the intervention group, except for the plasma therapy. No adverse effects or allergic responses associated with plasma transfusion were observed in recipients. The study results indicated that transfusion of convalescent plasma contributed to improvement of the patients' clinical outcomes including a decrease in the length of hospital stay, need for non-invasive mechanical ventilation and intubation and finally mortality rate which was significantly lower in the cases with less severe ARDS (PaO_2/FiO_2 above 200). This could suggest that convalescent plasma therapy could be more beneficial if administered in early stage of the disease and before the patient is critically ill. The number of deaths and discharged COVID-19 patients with severe ARDS (PaO_2/FiO_2 below 100) who were critically ill was equal in both intervention and control groups of the present study, strengthening the hypothesis of CP effectiveness in the less advanced stages of the disease. This is in accordance with the findings of the study done by Zeng et al. (2020) in which plasma transfusion in critically ill COVID-19 patients did not help with decreasing mortality (5

out of 6 patients died) [33]. The reason might lie in macrophage activation. Studies suggest that COVID-19 patients might undergo a macrophage activation associated with innate immune cells migration to lung tissues causing inflammation and pulmonary damage [18]. Inhibition of this immunological pathway might be helpful in averting cytokine storm and lung damage. This was also supported by a recent study reporting an up regulation of chemokines for innate immune cells mainly taking place within the first 7 days of the infection onset in COVID-19 patients [34]. The same pattern was observed in severe acute respiratory infections caused by other viruses such as H1N1 and SARS-CoV in which CP was transfused early after symptoms onset resulted in a reduction in mortality compared with those who received placebo or no therapy [22]. Moreover, a difference in case-fatality rate was observed in H1N1-infected patients who received CP earlier compared to those who received it later in their disease course [35]. Therefore, CP transfusion in early stages of COVID-19 disease may inhibit innate immune cells migration and lung damage.

The mean interval time from admission to plasma transfusion in the present study was 4.41 days with a minimum and maximum of 3–11 days. In a recent similar study done by Abolghasemi et al. [36] in which plasma transfusion was performed within three days of hospitalization, higher percentage of the patients (98.2%) receiving CP were recovered and discharged in total, compared with the present study (78.1%). This could be due to earlier administration of plasma and also less severity of the cases. Considering the patients' mean and maximum of SpO2 which were 85.95% and 93% in the abovementioned study [36], respectively, the patients included in the present study were more severe in comparison (SpO2 $\leq$ 85% and all the patients were suffering from ARDS). In this regard, the results of the present study also showed that all the patients (100%) with mild ARDS (200 $<$ PaO_2/FiO_2 $\leq$ 250) who received plasma therapy, were recovered and discharged which was statistically higher than the control group (73.7%), suggesting CP therapy is highly efficient in this group. However, it is worthy to mention that generally, a majority of COVID-19 patients with a mild and moderate course of disease survive without any intervention or by the mere use of supportive treatments currently recommended and available. Considering that plasma therapy does not seem to be significantly effective in critically ill patients according to the physiopathology of COVID-19, therefore, severe cases should be prioritized over critical and also mild/moderate ones in order to improve outcomes and decrease mortality rate. Accordingly, convalescent plasma transfusion seems to be most effective for potentially critical patients in the earlier stage of the disease with mild or moderate ARDS, before it progresses to critical state. This is where prognostic factors and also constant monitoring of the patients could substantially help with timely identification of the patients in need of plasma therapy and early administration of CP. The study results also showed a statistically significant decrease in APACHE II scores as well as the decrease in the disease severity in terms of SOFA score and increase in PaO_2/FiO_2 which was in accordance with the study done by Shen e al. [11]. However, no control group was included in the abovementioned study for comparing the outcomes. Among the studies published on the efficacy of CP for SARS-CoV-2 infection so far, not being a randomized clinical trial or not having control groups at all seems to be one of the most important limitations [11,14,33,36–39] According to a living systematic review (updated on Oct 12, 2020 for the second time) which included the results of 19 CP studies for treatment of COVID-19 patients, no certainty in CP effectiveness and safety could yet be concluded in this regard due to study limitations such as absence of a control group, results inconsistency, high risk of bias and low quality of reports [40]. Consequently, well designed controlled studies were recommended.

The present study was not designed as a randomized clinical trial either which might be considered as the most important limitation of this research. Following the standards and guidelines defined by Iran Ministry of Health and Medical Education applied in the center of our study, physicians must resort to additional treatment modality as well as standard care for all severe COVID-19 patients. According to the fact that all the patients included in the present study were severely ill with ARDS (SpO2 $\leq$ 85%, PaO_2/FiO_2 $\leq$ 250), ethical consideration prevented the physicians and researchers from depriving the patients of receiving an additional treatment modality. Therefore, designing a randomized clinical trial was not possible. Another limitation might be associated with continuing regular treatment including antivirals along with CP which could not be stopped due to the same considerations. However, we tried to make up for these limitations by assigning a historical control group with similar demographic characteristics, treatment regimen and disease severity, chosen randomly using the search tool of the matching system done by a blinded technician, in order to reduce the biases and provide a rather fair comparison. The study included more patients (64 patients) comparing with other similar studies to diminish the limitations of the small sample size, however; more clinical trials with higher number of patients with different disease severity are recommended to be carried out in order to assess CP efficacy in SARS-CoV-2 infection more accurately.

Authorship

A.A., M.S-S and N.S. designed the study and supervised; N.A., P.A. and A.AK. collected data; H.H.M. and M.E. performed statistical analysis; N.S. and M.S-S interpreted data and drafted the manuscript. A.A., M.S-S, N.S., M.M., S.A.J., M.A., M.M., Z.J., SH.A, A.S., M.M.N, H.R., A. B., Z.M., M.K., S.A. and S.H. contributed to administrative, technical and material support; S.E.H-A and M.A were members of advisory committee; all authors reviewed the manuscript and approved the final version.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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