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Research paper

Hyperimmune anti-COVID-19 IVIG (C-IVIG) treatment in severe and critical COVID-19 patients: A phase I/II randomized control trial

Shaukat Ali, PhD[a,*], Syed Muneeb Uddin[a], Elisha Shalim[a], Muneeba Ahsan Sayeed[b], Fatima Anjum[c], Farah Saleem[a], Sheikh Muhammad Muhaymin[a], Ayesha Ali[a], Mir Rashid Ali[a], Iqra Ahmed[a], Tehreem Mushtaq[a], Sadaf Khan[a], Faisal Shahab[a], Shobha Luxmi[d], Suneel Kumar[a], Habiba Arain[a], Mujtaba Khan[a], Abdul Samad Khan[e], Hamid Mehmood[d], Abdur Rasheed[f], Ashraf Jahangeer[g], SaifUllah Baig[d], Saeed Quraishy[h]

[a] Dow College of Biotechnology, Dow University of Health Sciences - Ojha Campus, Karachi, Pakistan
[b] Sindh Infectious Disease Hospital and Research Center, Karachi, Pakistan
[c] Dow Research Institute of Biotechnology and Biomedical Sciences, Dow University of Health Sciences - Ojha Campus, Karachi, Pakistan
[d] Dow University Hospital, Dow University of Health Sciences - Ojha Campus, Karachi, Pakistan
[e] National Control Laboratory for Biologicals, Islamabad, Pakistan
[f] Department of Research, Dow University of Health Sciences, Karachi, Pakistan
[g] Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan
[h] Dow University of Health Sciences - Ojha Campus, Karachi, Pakistan

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ABSTRACT

Background: Hyperimmune anti-COVID-19 Intravenous Immunoglobulin (C-IVIG) is an unexplored therapy amidst the rapidly evolving spectrum of medical therapies for COVID-19 and is expected to counter the three most life-threatening consequences of COVID-19 including lung injury by the virus, cytokine storm and sepsis.

Methods: A single center, phase I/II, randomized controlled, single-blinded trial was conducted at Dow University of Health Sciences, Karachi, Pakistan. Participants were COVID-19 infected individuals, classified as either severely or critically ill with Acute Respiratory Distress Syndrome (ARDS). Participants were randomized through parallel-group design with sequential assignment in a 4:1 allocation to either intervention group with four C-IVIG dosage arms (0.15, 0.20, 0.25, 0.30 g/kg), or control group receiving standard of care only (n = 10). Primary outcomes were 28-day mortality, patient's clinical status on ordinal scale and Horowitz index (HI), and were analysed in all randomized participants that completed the follow-up period (intention-to-treat population). The trial was registered at clinicaltrials.gov (NCT04521309).

Findings: Fifty participants were enrolled in the study from June 19, 2020 to February 3, 2021 with a mean age of 56.54±13.2 years of which 22 patients (44%) had severe and 28 patients (56%) had critical COVID-19. Mortality occurred in ten of 40 participants (25%) in intervention group compared to six of ten (60%) in control group, with relative risk reduction in intervention arm I (RR, 0.333; 95% CI, 0.087−1.272), arm II (RR, 0.5; 95% CI, 0.171−1.463), arm III (RR, 0.167; 95% CI, 0.024−1.145), and arm IV (RR, 0.667; 95% CI, 0.268−1.660). In intervention group, median HI significantly improved to 359 mmHg [interquartile range (IQR) 127−400, P = 0.009)] by outcome day, while the clinical status of intervention group also improved as compared to control group, with around 15 patients (37.5%) being discharged by 7th day with complete recovery. Additionally, resolution of chest X-rays and restoration of biomarkers to normal levels were also seen in intervention groups. No drug-related adverse events were reported during the study.

Interpretation: Administration of C-IVIG in severe and critical COVID-19 patients was safe, increased the chance of survival and reduced the risk of disease progression.

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* Corresponding author at: Dow University of Health Sciences., KDA Scheme 33, Gulzar-e-Hijri, Suparco Road Ojha Campus, Karachi -75270, Pakistan.
E-mail address: ali.shaukat@duhs.edu.pk (S. Ali).

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Research in context

Evidence before this study

Several studies were published during the COVID-19 pandemic to assess whether convalescent plasma or intravenous immunoglobulin (IVIG) transfusion is effective and safe in the treatment of COVID-19 patients. We performed a literature search using PubMed, medRxiv, Web of Science, Google Scholar and Scopus upto March 30, 2021 with the following search terms: Convalescent plasma, Randomized clinical trial, Hyperimmune anti-SARS-CoV-2 Intravenous Immunoglobulin, COVID-19. We included randomized control trials and full-text manuscripts available in the English language. Previous studies suggested that convalescent plasma (CP) and IVIG can work effectively in improving survival rates and reducing disease progression in emerging viral infections, including Corona related SARS-CoV-1 infection (SARS) and Middle East respiratory syndrome (MERS). The benefit of CP with particular focus to hyperimmune IVIG for COVID-19 has not been established yet and was short of statistical significance in a small, randomized study of severe and critical COVID-19 patients.

Added value of this study

This is the first report of hyperimmune anti-COVID-19 Intravenous Immunoglobulin, C-IVIG, tested in severe and critical COVID-19 patients. We used a phase 1/2 study design to assess the safety and efficacy of four different C-IVIG doses (0.15 g/kg, 0.2 g/kg, 0.25 g/kg, and 0.3 g/kg) compared to a control group. The patients who received C-IVIG with standard of care had reduced risk of mortality than those in patients who received standard of care only.

Implications of all the available evidence

Patients classified as having severe or critical COVID-19 have a higher risk of mortality, especially those with underlying comorbidities. Our findings indicate that C-IVIG is well tolerated and increase the chance of survival while reducing the risk of disease progression. Further studies of the safety and effectiveness of this treatment are needed.

1. Introduction

Coronavirus disease 2019 (COVID-19) was declared as a pandemic by the World Health Organization on March 11, 2020 [1]. It is a systemic disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Globally, over 121.3 million confirmed cases of COVID-19 have been reported till March 17, 2021 including 2.6 million deaths and an average of over 400,000 new cases daily [2]. COVID-19 is categorized as a biphasic illness with relatively mild protean phase directed by viral replication, and second phase, directed by the host immune response [3]. This second phase may lead to severe and critical COVID-19 cases progressing towards a life-threatening multiple organ dysfunction, characterized by refractory hypoxemia due to acute respiratory distress syndrome (ARDS) [4]. Therefore, from a pathophysiological viewpoint, clinically significant treatments for COVID-19 will possibly evolve from immunomodulation. Currently, there is no consensus on treatment algorithms for COVID-19, as the evidence available is not well controlled and largely anecdotal. Given the rapid and catastrophic spread of COVID-19, there is an urgent need for effective therapeutic options while novel therapies and vaccines are being developed and explored.

Intravenous immunoglobulin (IVIG), has been used as a therapeutic agent against a variety of inflammatory, infectious, autoimmune, and viral diseases including SARS and Middle East respiratory syndrome (MERS) [5,6]. This study explores Hyperimmune anti-COVID-19 Intravenous Immunoglobulin (C-IVIG), prepared using pooled high titer convalescent plasma (cut-off index >10) obtained from COVID-19 recovered individuals [7]. C-IVIG when infused in COVID-19 patients, is expected to regulate disease progression via multiple mechanisms including SARS-CoV-2 neutralization, immunomodulation to prevent cytokine storm, and prevention of superimposed bacterial infection (sepsis) due to presence of polyclonal antibodies against other endemic pathogens [8,9,10].

Despite great interest, lack of availability of clinical evidence for the safety and efficacy of the therapy has limited the use of hyperimmune intravenous immunoglobulin as one of the first-line therapeutic options against COVID-19. In this context, Phase I/II, single center, single-blinded and randomized-controlled trial was carried out to investigate the safety and clinical efficacy of C-IVIG in severe and critically ill COVID-19 patients.

2. Methods

2.1. Study design

This was a single center, phase I/II, randomized controlled, single-blinded trial, conducted between June 19, 2020, and February 3, 2021 through parallel-group design with sequential assignment. The trial has been completed and registered at https://clinicaltrials.gov (NCT04521309) [11]. Original and final study protocol is included in supplementary materials with summary of changes.

The trial has been approved by the Institutional Review Boards (IRB) of Dow University of Health Sciences (IRB-1685/DUHS/Approval/2020/), National Bioethics Committee (No.4−87/NBC-471-COVID-19−07/20/), and regulated independently by the national drug safety monitoring board.

2.2. Participants

Written informed consent was obtained from each patient or from the patient's authorized representative if the patient was unable to provide consent, and were randomized either to receive C-IVIG with standard of care (SOC) or only SOC. All participants were laboratory-confirmed COVID-19 infected individuals admitted to the clinical trial site approved tertiary care hospital (Sindh Infectious Diseases Hospital & Research Center, Dow University Hospital) in Karachi, Pakistan. Participants were classified as either severely (hospitalized, requiring any supplemental oxygen) or critically (hospitalized, requiring non-invasive ventilation, high-flow oxygen devices or invasive ventilation) ill with Acute Respiratory Distress Syndrome (ARDS) i.e. dyspnea, respiratory rate ≥30/min, blood oxygen saturation ≤90%, PaO_2/FiO_2 <300, and lung infiltrates >50% on chest X-ray [12]. Participants with a history of IgA deficiency, autoimmune disorder, thromboembolic disorder, and allergic reaction to immunoglobulin treatment were excluded from the study. Similarly, pregnant females, patients requiring two or more inotropic agents to maintain blood pressure and patients with acute or chronic kidney injury/failure were also excluded.

2.3. Randomization and masking

All participants were blinded and attended a single study site following enrollment. Eligible participants were randomly assigned in 4:1 ratio (40 tests: 10 controls) by sequentially numbered opaque sealed envelope simple randomization method, either to receive C-IVIG plus SOC (intervention group), or only SOC (control group). A randomization list was generated by a hospital personnel unrelated

to this study while the study personnel were unaware of the sequence of assignment. At the time of randomization, the study personnel received a sealed opaque envelope with assignment to intervention or control group. The intervention group was sequentially randomized into four intervention arms (10 participants in each arm) receiving four different concentrations of 5% C-IVIG doses: Arm I: 0.15 g/Kg with SOC, Arm II: 0.2 g/Kg with SOC, Arm III: 0.25 g/Kg with SOC, and Arm IV: 0.3 g/Kg with SOC.

2.4. Intervention

C-IVIG is a preparation of hyperimmune polyclonal immunoglobulin fractionated from pooled convalescent plasma of recovered COVID-19 individuals, asymptomatic for more than 15 days. A total of 203 participants were screened, of which 173 were selected for convalescent plasma donation. Plasma donors with variable titers contributed to the pool, however a lower limit of 10 cut-off index (COI) was established by measurement through electrochemiluminescence immunoassay analyzer (ECLIA). The variable titer of convalescent plasma donors led to the variable titer of pooled plasma, and subsequently variable anti-SARS-CoV-2 antibody level of up to 104± 30 COI measured through ECLIA [7]. Patients infused with C-IVIG followed a pre-infusion protocol and were given methylprednisolone (40-mg) I.V. and adequate hydration was ensured. The infusion protocol is explained in detail in additional methods in supplementary appendix. All participants, irrespective of their group assignment, received SOC according to the national clinical management guideline for COVID-19 Infection which includes airway support, antiviral medications, anticoagulant, steroid, hemodynamic support and antibiotics when required [12]. SOC included Remdesivir (200 mg loading then 100 mg once daily for 5 days), Enoxaparin and corticosteroids, dexamethasone (6 mg once daily) or Methylprednisolone (0.5−1 mg/kg twice daily) initiated at the time of hospitalization till resolution of ARDS.

2.5. Clinical outcomes

Study participants were followed after study enrollment on prespecified days to assess safety and efficacy of C-IVIG treatment. Day of the patient's death or discharge from hospital was established as outcome day. A pre-designed data collection form was used for collection of demographic, clinical and laboratory data for each participant. Reports were obtained using the hospital's centralized record database software, Health Management Information System (HMIS), and records maintained by the hospital staff. The obtained reports were analyzed by treating physicians and research personnel.

Primary outcomes of the study include 28-day mortality, patient's clinical status during study duration and Horowitz index at outcome day. Horowitz index calculated using PaO_2/FiO_2 ratio has been used to assess the severity of ARDS in the patients [13]. Patients' clinical status was assessed on seven-category ordinal scale and was recorded on the specific observation days. The ordinal scale had following seven categories: 1, not hospitalized and no limitations of activities; 2, not hospitalized, with limitation of activities, home oxygen requirement, or both; 3, hospitalized, not requiring supplemental oxygen; 4, hospitalized, requiring any supplemental oxygen; 5, hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices; 6, hospitalized, receiving invasive mechanical ventilation; and 7, death.

Secondary outcome measures include days to discharge from hospital, days to death, days to negative SARS-CoV-2 PCR, days to invasive ventilation, days to improvement in ordinal scale by 3 categories, change in C-reactive protein (CRP) levels, radiological changes in patient's X-ray, change in Ferritin, and Lactate Dehydrogenase (LDH) level.

Safety outcomes were reported in terms of adverse events (enrollment to outcome day) and immediate adverse events (occurring within 24hours of enrollment). Other measures to assess safety included routine vitals measurement during hospital stay, and assessing laboratory parameters like Liver Function Test (LFT), Procalcitonin, Sodium, Potassium, Chloride and Bicarbonate levels during hospital stay from day of enrollment till 28th day after enrollment. Complete data collection and follow-up plan is shared in supplementary appendix.

2.6. Statistical analysis

This phase I/II trial was approved for a sample size of 50 participants by national ethical and regulatory bodies and no formal sample size calculation was performed due to unknown outcome proportions. All randomized study participants were included in intention-to-treat population and all participants completing the study period were analyzed (complete case analysis). Normality of continuous data was checked by Shapiro-Wilk test and non-parametric tests were applied for analysis of clinical parameters. Continuous variables are presented as Mean (±S.D) or Median [Interquartile range (IQR)], and categorical variables are presented as percentages.

In the primary analysis strategy, we used the Kaplan−Meier curve (Breslow test) that compares the time to reach the primary end point in the trial groups. Significance testing of primary and secondary outcomes for assessment of safety and efficacy parameters was conducted by Mann-Whitney U test. Categorical data was compared with Chi-square and value of two sided Fisher exact test was recorded. An estimate of the relative risk (RR) and 95% confidence interval is also reported. No interim efficacy review was done and analysis of study results was done after the statistical analysis plan was finalized. Statistical analysis was done using SPSS software, version 24.0 and *P*-values <0.05 is considered statistically significant. All P-values are two-sided and are shown without adjustment for multiple testing. All primary outcomes were analyzed completely with no missing data, however complete case analysis was performed for the analysis of the secondary outcomes without imputation for missing data. Secondary outcome measures with missing data have not been reported in the manuscript, and do not influence the analysis of other outcomes. Data of such parameters have been presented in supplementary appendix.

3. Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, report writing, and decision to submit for publication, as well as do not have access to dataset. Dr Shaukat Ali as principal investigator and corresponding author had access to final dataset and made final decision to submit for publication.

4. Results

Between June 19, 2020, and February 3, 2021, a total of 70 patients were assessed as study participants. After excluding 20 patients from the study due to multiple reasons (13 did not fall into eligibility criteria, 7 declined to participate), 50 patients (70% male) falling in inclusion criteria were randomized into four arms (n = 10 each) of intervention group, and a control group (n = 10) through parallel-group design with sequential assignment (Fig. 1).

Detailed demographics and baseline clinical characteristics of all study participants has been summarized in Table 1 and S1. Mean age of patients in the study was 56.54±13.17 years with more than half of the patients having comorbidities (70%), mainly hypertension (52%) and diabetes (36%). The mean(±SD) time from the onset of COVID-19 symptoms to enrollment was recorded as 8.0 ± 3.08 and

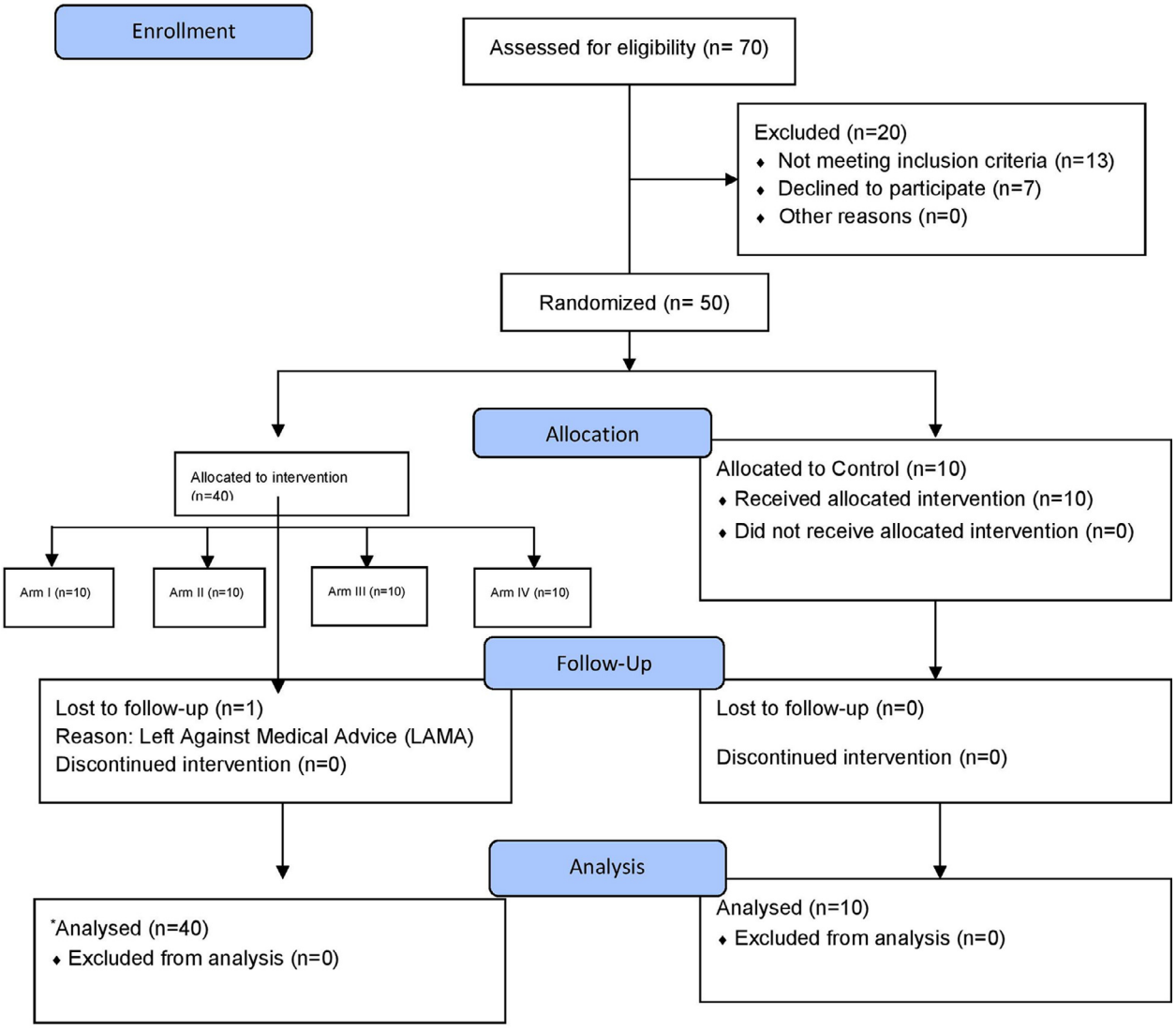


Fig. 1. Trial Profile.

8.37±3.14 days for control and four C-IVIG intervention arms, respectively. Clinical status of the patients was estimated according to ordinal scale; 44% of the patients were found to be severe (category 4) and 56% were critical (54% in category 5 and 2% in category 6).

A total of 16 (32%) patients died within 28 days of enrollment in both the groups. Of the 40 patients in intervention group 10 (25%) died (RR, 0.417; 95% CI, 0.199–0.871) while of 10 patients in control group 6 (60%) patients died. When compared to control group, there was a relative risk reduction in intervention arm I (RR, 0.333; 95% CI, 0.087–1.272), intervention arm II (RR, 0.5; 95% CI, 0.171–1.463), intervention arm III (RR, 0.167; 95% CI, 0.024–1.145), and intervention arm IV (RR, 0.667; 95% CI, 0.268–1.660). Comparison of the survival distribution among all groups were assessed by Breslow test, and it was found that patients in intervention group had better survival distribution when compared to control group, with significant survival distribution difference in arm 1 ($P = 0.048$) as shown in Fig. 2 Comparison of 28-day mortality among different intervention arms showed no statistical significance in any of the C-IVIG dosages used. Severe COVID-19 patients showed a significant reduction in mortality ($P = 0.002$) when compared to critical COVID-19 patients, with mortality in 10 out of 23 (43.5%) critical patients and none among the 17 severe patients (data not shown).

On the outcome day, median Horowitz index (HI) was significantly improved to 393 mmHg [interquartile range (IQR), 124.75–441.5; $P = 0.009$] in arm I and to 361.5 mmHg (IQR, 309.25–427.25; $P = 0.022$) in arm III when compared with the control group (105 mmHg; IQR, 73.5–319.5) (Table 2). In all intervention arms, median HI significantly improved to >300 mmHg (non ARDS) on outcome day as compared to the control group with a median HI of 105 mmHg ($P = 0.34$) as shown in Table S6.

Clinical status at 7th day of enrollment according to ordinal scale was observed in all groups (Fig. 3). On 7th day of enrollment, no patient was discharged from the control group, while 5 of 10 patients (50%) in arm III, 4 of 10 patients (40%) in arm I, and 3 of 10 patients (30%) in arm II and IV were discharged with no limitation of activities. By 7th day, mortality (category 7) was observed in 3 controls (30%), 2 (20%) patients in arm I and in 1 (10%) patient in arm IV. While no mortality was observed in arms II and III by 7th day. Overall, none of

Table 1
Demographic, and Baseline Clinical Characteristics of Participants.

Variables/Parameters	Control (n = 10)	Intervention groups (n = 40)	Arm I	Arm II	Arm III	Arm IV
Age (years), Mean ± S.D	59.1 ± 12.06	55.9 ± 1.34	47.06±8.75	67.4 ± 9.17	54.14±14.46	55.3 ± 13.9
Gender, n (%)						
Male	7 (70)	28(70)	7 (70)	10 (100)	5 (50)	6 (60)
Female	3 (30)	12(30)	3 (30)	0	5 (50)	4 (40)
Days from onset of symptoms, Mean ± S.D	8 ± 3.08	8.37±3.14	10±2.90	8 ± 2.05	7 ± 4	8.5 ± 2.9
Comorbidities, n (%)						
Diabetes	4(40)	14(35)	3(30)	6(60)	4(40)	1(10)
Hypertension	4(40)	22(55)	6(60)	5(50)	6(60)	5(50)
Chronic lung disease	0	5(12.5)	3(30)	0	0	2(20)
Cardiac disease	1(10)	3(7.5)	1(10)	2(20)	0	0
Hepatitis C/Chronic liver disease	0	0	0	0	0	1
Clinical status by ordinal scale n (%)						
4. Hospitalized, requiring any supplemental oxygen (severe)	5 (50)	17(42.5)	5(50)	5(50)	4(40)	3(30)
5. Hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices	5 (50)	22(55)	4(40)	5(50)	6(60)	7(70)
6. Hospitalized, receiving invasive mechanical ventilation	0	1(2.5)	1(10)	0	0	0
Medication during trial, n (%)						
Remdesivir	10(100)	37(92.5)	7(70)	10(100)	10(100)	10(100)
Antibiotics	10(100)	40(100)	10(100)	10(100)	10(100)	10(100)
Steroids	10(100)	40(100)	10(100)	10(100)	10(100)	10(100)
Tocilizumab	2(20)	1(2.5)	1(10)	0	0	0
Median (IQR) Horowitz Index	92(68.75–124.2)	89.5(69.25–143.75)	100.5(63–163)	101.5(68.75–130.25)	81(75.25–154)	80(57.25–129.25)
Median (IQR) C-Reactive Protein	104.05(79.85–141.46)	99.9 (46–183.4)	108.3(42.12–149.54)	81.83(38.07–190.27)	100.35(47.12–178.9)	162.27(48–212.17)

the patients in the intervention group classified in category 4 (severe patients) at enrollment proceeded to category 5 of ordinal scale, compared to 2 (40%) of 5 patients in the control group. Furthermore, of the 22 patients in category 5 in intervention group, 10 (45.5%) progressed to category 6, compared to 4 (80%) of 5 patients in the control group.

Highest decrease in the CRP levels of patients was observed in arm IV after 24 h of enrollment with the mean(±SD) value of 87.35(±6.30) mg/L, compared to 18.31(±5.36) mg/L in the control group (Table 3). There was a decrease in median days to hospital discharge in arms I (5.5; IQR, 4.25–11.75), III (6; IQR, 4.5–11) and IV (7.5; IQR, 5.5–9) compared to the control group (8; IQR, 8–8.75). Median days to hospital discharge was significantly reduced (P = 0.002) in severe COVID-19 patients (5; IQR, 4–7.5) when compared to critical COVID-19 patients (11; IQR, 7.5–14.5). Median days in which mortality occurred from day of enrollment was 26 days in arm III, which was significantly prolonged in comparison to the control group which was 9 (5.5–19) days. None of the patients in the arm I required invasive ventilation, whereas, in the control group the median days of invasive ventilation was 8 (2–8.5) days. Arms III and IV with a median of 4 days (2.75–6) were found effective in reducing duration of improvement in ordinal outcomes by 3 categories.

Of the 40 patients in intervention group chest X-rays showed improvement in 23 (57.5%) compared to 7 (70%) in control group, while it worsened in 10% of patients in intervention group in comparison to 20% in control group. Chest X-rays of 14 patients showed no significant improvement or worsening (Figure S5). Although clinically insignificant different from control group, Ferritin and LDH levels of arm III were improved when observed on the outcome day (Table S5, S6 and Figure S2).

As judged by the treating physicians on the basis of known IVIG adverse events, baseline biomarkers and timing of adverse event, none of the patients experienced drug related serious adverse events. The percentage of patients who had an adverse event during treatment was 70% (28 of 40) in the intervention group and 70% (7 of 10) in the control group. There were a total of 73 adverse events (51 in intervention, 22 in control group), one of which (chills) occurred within 24 h of infusion (Table S2, S3 and S4). No major changes in vital signs were observed during infusion, and the infusions were completed in all instances without any pause.

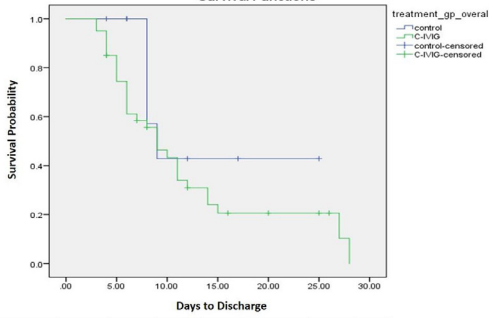
5. Discussion

This study is the first report of usage of hyperimmune anti COVID-19 Intravenous Immunoglobulin (C-IVIG) prepared from convalescent plasma [7] to evaluate its safety and efficacy in severe and critical COVID-19 patients. The use of C-IVIG to treat COVID-19 was found safe as no immediate or serious drug related adverse event was reported in any patient of intervention arms.

The exploration of passive immunization as treatment modality for COVID-19 patients has been limited to the use of convalescent plasma [14,15] and SARS-CoV-2 neutralizing monoclonal antibody preparations to date [16,17]. Many systematic reviews, meta-analysis and clinical studies [18-20] had discussed and looked forward to reports on usage of hyperimmune antibody drugs for treatment of COVID-19 patients. C-IVIG showed a relative risk reduction in the 28-day mortality rate in all intervention arms as compared to the control group, however statistically insignificant. Excessive inflammation is one of the major causes of COVID-19 pathology, and severe cytokine storm has been found to be associated with an increased death rate in critical COVID-19 patients [21]. Anti-cytokine effects, inhibition of complement activation, and down-regulation of B and T cells' functions by IVIG can prevent organ failure and subsequent mortality [22,23]. The trial included both severe and critical patients, however results showed a significantly better recovery in terms of survival, reduction in disease severity and hospital stay when C-IVIG was infused in severe patients. This study corroborates reports [17,24] which suggested that antibody-based interventions work better when administered early in the course of the COVID-19.

The median days to discharge post C-IVIG infusion was 8 days compared to Dexamethasone treatment (12 days), Remdesivir treatment (10 days), Tocilizumab treatment (6 days) and convalescent

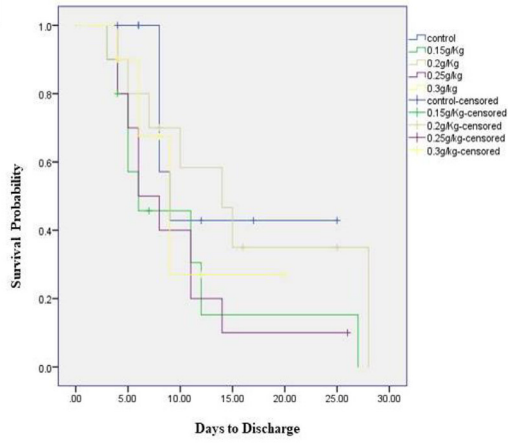
(A)



At risk	0	5	10	15	20	25	30
CONTROL	10	10	4	3	2	1	0
TEST	40	32	15	7	5	4	0

# censored subjects		P value
Control	6	
Intervention Groups	10	0.151

B



At risk	0	5	10	15	20	25	30
Control	10	10	4	3	2	1	0
Arm I	10	7	4	2	2	2	0
Arm II	10	9	6	4	3	2	0
Arm III	10	8	5	2	2	2	0
Arm IV	10	10	5	2	1	0	0

# censored subjects		p value
Control	6	N/A
Arm I	2	0.048*
Arm II	3	0.770
Arm III	1	0.060
Arm IV	4	0.389

Fig. 2. (A) Comparison of days to discharge between control group (n = 10) and intervention groups (n = 40); (B) all study groups (n = 10 in each group).

plasma treatment (11 days) [25-28]. A shorter discharge time means minimum utilization of hospital resources, such as critical care resources, which is essential during this COVID-19 pandemic to relieve the burden on the healthcare system. The high levels of biomarkers (CRP, LDH, Ferritin and IL-6) are positively associated with COVID-19 disease severity [29], and reduction of these biomarkers post-infusion suggested anti-inflammatory effect of C-IVIG. Portable chest X-rays have proven to be one of the most common diagnostic tools for detection of COVID-19, however it's prognostic value in COVID-19 pneumonia is yet to establish. [30]. As observed in some cases of this study, although the X-rays showed improvement, the overall disease severity of those patients increased.

As this study was aimed to observe the effects of C-IVIG including passive immunization and immunomodulation as treatment modality, some important outcome measures such as anti-SARS-CoV-2 antibody levels and IL-6 levels should have been reported. However, the facility of measuring these parameters was not available at the study center at the start of this clinical trial, and although the authors tried to collect samples to process them later, plenty of samples were either not collected or were contaminated which led to missing vital data (Available data provided in supplementary appendix: Figure S3). Moreover, this study included severe and critical COVID-19 patients, showing exacerbated symptoms and receiving multiple other treatments with their own respective side effects. These factors affected the analysis of adverse events by investigators to associate them with C-IVIG. Clinical efficacy of C-IVIG, although significantly better than standard of care, cannot be attributed solely to C-IVIG rather to its combination with standard of care.

One of the objectives of the phase I/II trial was to explore variable dosages for safety and efficacy, however no statistically significant difference was found among dosages when compared for primary outcomes (28-day mortality). There was significantly improved therapeutic response to C-IVIG treatment in severe COVID-19 patients in comparison to critical COVID-19 patients. Therefore the results from this study warrant the phase II/III clinical trial, with the lowest dosage (0.15 g/kg) of C-IVIG in severe COVID-19 patients, and higher study power for further evaluation of its safety along with efficacy analyzing 28-day mortality as primary outcome.

The study reports use of hyperimmune intravenous immunoglobulin prepared from convalescent plasma in treating severe and critical COVID-19 patients. Single dose of C-IVIG in combination with standard of care was found both safe and efficacious while increasing the chance of survival and reducing the risk of disease progression.

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Contributors

Ali, Shaukat (corresponding author) *Concept design, Supervision,* Investigation of results, Original Draft, Preparation *and finalization of manuscript.* Muneeb Uddin, Syed Study design, Data Collection and analysis, *drafting and finalization of manuscript.* Shalim, Elisha Data collection, data analysis, drafting, Editing and compilation of manuscript. Sayeed, Muneeba Ahsan Contributed in methodology, data analysis and manuscript drafting. Anjum, Fatima Contributed in Study design, methodology, statistical analysis, manuscript drafting and review. Saleem, Farah Contributed in methodology, statistical analysis and manuscript drafting. Muhammad Muhaymin, Sheikh Contributed in methodology, data validation, and manuscript drafting. Ali, Ayesha Contributed in methodology, and manuscript drafting, review and editing. Ali, Mir Rashid Contributed in Study Design, methodology, data collection and manuscript drafting. Ahmed, Iqra Contributed in methodology, statistical analysis and manuscript drafting. Mushtaq, Tehreem Contributed in methodology, data collection and manuscript drafting. Khan. Sadaf statistical analysis and manuscript drafting and review. Shahab, Faisal Contributed in methodology, data collection and manuscript drafting. Luxmi, Shobha Contributed in methodology, data collection and manuscript drafting. Kumar, Suneel Contributed in methodology, data collection and manuscript drafting. Habiba Contributed in methodology, data collection and manuscript drafting. Khan, Mujtaba Contributed in methodology, data collection and manuscript drafting. Khan, Abdul Samad Expert advice, study design, manuscript drafting and review. Mehmood, Hamid Contributed in methodology, data collection and manuscript drafting. Abdur Rasheed Statistical analysis and manuscript drafting.

Table 2
Comparison of the primary outcomes of intervention and control group.

Primary outcomes	Control (n = 10)	Intervention Arms (n = 40)	Relative Risk	[I]P-value	Arm I (n = 10)	Relative Risk	[II]P-value	Arm II (n = 10)	Relative Risk	[III]P-value	Arm III (n = 10)	Relative Risk	[IV]P-value	Arm IV (n = 10)	Relative Risk	[V]P-value
28-day Mortality, n (%)	6 (60)	10(25)	0.417 (0.199–0.871)	0.056	2 (20)	0.333 (0.087–1.272)	0.17	3 (30)	0.5 (0.171–1.463)	0.37	1 (10)	0.167 (0.024–1.145)	0.057	4 (40)	0.667 (0.268–1.660)	0.656
Median (IQR) Horowitz Index at outcome day	105 (73.5–319.5)	359 (127–400)		0.009	393 (124.75–441.5)	–	0.009*	359	(156–400)	–	0.54	361.5				
(309.25–427.25)	–	0.022*	332 (71–367)	–	0.204											
Clinical status at 7th day after enrollment by ordinal scale: n (%)																
1. Not hospitalized and no limitations of activities	0				4 (40)	–	0.087	3 (30)	–	0.211	5 (50)	–	0.033*	3 (30)	–	0.211
2. Not hospitalized, with limitation of activities, home oxygen requirement, or both	0				1 (10)	–	1.000	0	–	–	0	–	–	0	–	–
3. Hospitalized, not requiring supplemental oxygen	2 (20)				1 (10)	–	1.000	0	–	0.474	3 (30)	–	1.000	0	–	0.474
4. Hospitalized, requiring any supplemental oxygen	2 (20)				1 (10)	–	1.000	2 (40)	–	1.000	1 (10)	–	1.000	3 (30)	–	1.000
5. Hospitalized, requiring noninvasive ventilation or use of high-flow oxygen devices	3 (30)				1 (10)	–	0.582	5 (30)	–	0.650	0	–	0.211	2 (20)	–	1.000
6. Hospitalized, receiving invasive mechanical ventilation	0				0	–	–	0	–	–	1 (10)	–	1.000	1 (10)	–	1.000
7. Death	3 (30)				2 (20)	–	1.000	0	–	0.211	0	–	0.211	1 (10)	–	0.582

* $P < 0.05$ is considered as statistically significant.
[I] P-value= Control vs Intervention Arms.
[II] P-value= Control vs Arm I.
[III] P-value=Control vs Arm II.
[IV] P-value= Control vs Arm III.
[V] P-value= Control vs Arm IV.

Table 3
Comparison of secondary outcomes among intervention and control arm.

Variables	Control	Arm I	[II]P-value	Arm II	[III]P-value	Arm III	[IV]P-value	Arm IV	[V]P-value
Change in C-Reactive protein (CRP) level after 24 h of enrollment. Mean ± *S*.D (mg/L)	18.31±5.36	26.08± 3.62	0.825	58±7.74	0.331	45.45± 6.37	0.145	87.35 ± 6.30	0.149
Median number of Days (IQR):									
From enrollment to hospital discharge	8(8−8.75)	5.5(4.25−11.75)	0.493	10(5−15)	0.703	6(4.5−11)	0.48	7.5(5.5−9)	0.737
From enrollment to death	9(5.5−19)	5.5[α](4 −7)	0.399	16[α](8−25)	0.362	26[β]	0.13	10(5−18)	1
From enrollment to negative SARS-CoV-2 Polymerase Chain Reaction (PCR) test	15(4.2−49)	18(15−11.5)	0.806	11.5(7.25−20.25)	0.773	23(13.5−34.5)	0.829	5.5(2.75−7.75)	0.199
From enrollment to invasive ventilation	8(2−8.5)	N/A	0.203	7(2.25 −8.75)	0.208	8[β]	0.604	5(2.25−8.5)	0.83
From enrollment to improvement in clinical status by 3 categories	7.5(3.25−8)	5(4−11)	0.924	9(5−14)	0.344	4(2.5−6)	0.24	4(2.75−6)	0.161
Adverse events n (%)	7 (70)	7 (70)	1.000	8 (80)	1.000	5 (50)	0.650	8 (80)	1.000

[β] Frequency is mentioned instead of median (IQR). Median (IQR) could not be determinate.
[α] (range) is given instead of IQR. IQR could not be determinate.
[II] P-value= Control vs Arm I.
[III] P-value=Control vs Arm II.
[IV] P-value= Control vs Arm III.
[V] P-value= Control vs Arm IV.

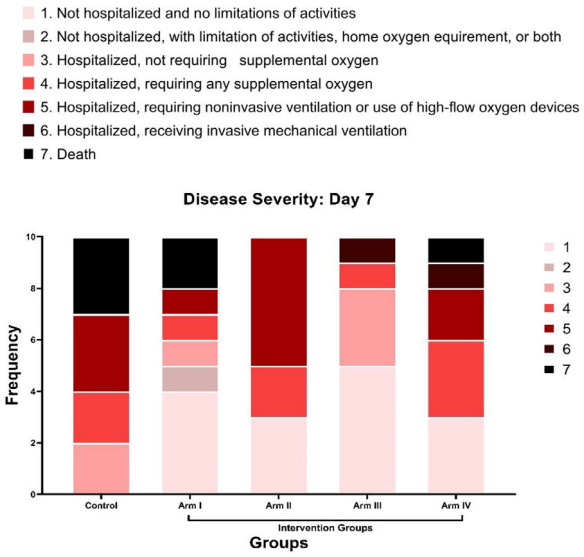

Fig. 3. Clinical status at day 7 according to seven-category ordinal scale.

Ashraf Jahangeer Statistical analysis and manuscript drafting. Baig, SaifUllah Contributed in methodology, data collection and manuscript drafting. Quraishy, Saeed Approval of the study design, data validation and manuscript finalization.

Data sharing

All study data including individual participant data (deidentified participant data) and a data dictionary defining each field in the set, will be made available to others. Additionally, statistical plan, study protocol and informed consent forms will also be available. Data will be available from publication till one year. Any request for the data could be sent to ali.shaukat@duhs.edu.pk.

Declaration of Competing Interest

Muneeba Ahsan Sayeed, Farah Saleem, Sheikh Muhammad Muhaymin, Sadaf Khan, Shobha Luxmi, Habiba Arain, Abdul Samad Khan, Hamid Mehmood, Abdur Rasheed, Ashraf Jahangeer, SaifUllah Baig, Saeed Quraishy declare they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eclinm.2021.100926.

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