An Investigation Into the Beneficial Effects of High-Dose Interferon beta 1-a, Compared to Low-Dose Interferon Beta 1-a (the base therapeutic regimen) in moderate to severe COVID-19

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Research

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) has been a serious obstacle in front of public health. Interferon-beta 1a (IFN-β 1a) has been used to treat patients with COVID-19. We aimed to compare the effectiveness of high dose IFN-β 1a compared to low dose IFN-β 1a (the base therapeutic regimen) in moderate to severe COVID-19 cases.

Methods: In this randomized, controlled, and clinical trial, eligible patients with confirmed SARS-CoV-2 infections were randomly assigned to receive one of the two following therapeutic regimens: The intervention group was treated with high dose IFN-β 1a (Recigen) (Subcutaneous injections of 88μg (24,000 IU) on days 1, 3, 6) + lopinavir /ritonavir (Kaletra) and the control group was treated with low dose IFN-β 1a (Recigen) (Subcutaneous injections of 44μg (12,000 IU) on days 1, 3, 6) + lopinavir /ritonavir (Kaletra) (400mg/100 mg twice a day for 10 days, orally, in all two groups).

Result:

A total of 168 COVID- 19 confirmed patients underwent randomization; 83 were assigned to the intervention group and 85 were assigned to the control group. Median Time To Clinical Improvement (TTIC) for cases treated with low dose of IFN-β1a was shorter than that for cases treated with high dose of IFN-β1a (6 vs10 days; P=0.018). Hazard Ratio for TTCI in the Cox regression model was 1.56 (95% CI: 1.05-2.30, P-value=0.026). Due to differences between some baseline clinical factors between intervention and control group, we; therefore, performed an adjusted analysis by including spo2, D-dimer and CRP in Cox regression model. The model failed to reach a significant difference between two groups. The adjusted HR was 1.37 (95% CI: 0.88-2.12, P-value=0.16). No difference was observed in terms of mortality between two groups.

Conclusion

The use of high-dose IFN-β 1a did not improve TTCI in hospitalized patients with moderate to severe COVID-19. Also, it has not any significant effect in mortality reduction compared with treating with low-dose IFN-β 1a.

Trial registration: The trial was confirmed by the Ethics in Medical Research Committee of the Shahid Beheshti University of Medical Sciences. signed informed consents were obtained from all the participants or their legally authorized representatives. This trial has been registered as ClinicalTrials.gov, NCT04521400.

Introduction

Coronavirus disease 2019 (COVID-19) has been a serious obstacle in front of public health. Exponential growth of infected cases with COVID-19 has prompted World health organization (WHO) to declare a

pandemic situation on March 11th, 2020. As of 18 November, more than 55 million confirmed cases of COVID-19 and 1,328,000 deaths were reported in the world (1).

This virus was also named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) because genome analysis indicates that the new Coronavirus is the same subgenus as the SARS virus but in a different clade(2).

Cytokine storm, surge in cytokines as a result of an imbalance between inflammatory and anti-inflammatory cytokines, has been reported by pathophysiology studies in various cases with COVID-19 (3). It is responsible for multi-organ failure and high mortality rate in COVID-19 cases with pneumonia (4). Therefore, immunomodulatory agents are an option for the treatment of COVID-19. Of all immunomodulatory agents, interferons (IFNs) exert a broad range of influences on immune system including antiviral, antiproliferative and immunomodulatory activities (5). IFNs, a group of soluble glycoproteins, are produced by certain cells in response to virus, bacteria, and tumor cells(6).

IFNs are segregated into three major types including type I IFN (mainly alpha and beta), type II IFN(gamma) and type III IFN (lambda) (7). It has been demonstrated that IFN-β is more useful in inhibiting coronaviruses as opposed to IFN-α. IFN-β 1a and IFN-β 1b have the most significant role against SARS-CoV and MERS-CoV (8, 9). Previous in vitro and in vivo studies showed beneficial effect of IFN-B 1a against coronavirus including avian infectious bronchitis virus, murine hepatitis virus and SARS-CoV (10). In recent in vitro study, the usefulness of IFN-β 1a in reduction of SARS-CoV-2 replication has been shown (11). Although the effect of IFN administration in COVID-19 pharmacotherapy has been investigated, there is no recommendation on proper dosage of INF in the treatment of COVID-19.

Owing to the absence of reliable treatment for COVID-19, intensive efforts are being made to identify promising antiviral drugs against COVID-19. In this study, we; therefore, performed a single-center, randomized, open-label, controlled trial to investigate the efficacy and safety of high-dose IFN-β 1a in combination with lopinavir/ritonavir compared with low-dose IFN-β 1a in combination with lopinavir/ritonavir (the base therapeutic regimen) in moderate to severe COVID-19 patients.

Material And Methods

Trial Design and Oversight

In this single-center, open-label, randomized, controlled, parallel-group and clinical trial, eligible patients with confirmed SARS-CoV-2 infections were randomly assigned in a 1:1 ratio to receive one of the two following therapeutic regimens: 1) IFN-β1a (Recigen) (Subcutaneous injections of 88 µg (24,000 IU) on days 1, 3, 6) + lopinavir/ritonavir (Kaletra) [intervention group], 2) IFN-β1a (Recigen) (Subcutaneous injections of 44 µg (12,000 IU) on days 1, 3, 6) + lopinavir/ritonavir (Kaletra) (400 mg/100 mg twice a day for 10 days, orally, in all two arms) [control group]. Intervention and control groups received standards of care including necessary oxygen support and non-invasive or invasive mechanical ventilation. The study was commenced at August 20th and terminated at September 4th, 2020 at Loghman Hakim hospital.

We tried to collect our data on a potential treatment regimen by performing a pragmatic randomized controlled trial for moderate to severe COVID-19 patients without sacrificing any critical investigational component in a reasonable time frame.

Due to emergency situation as well as brisk rate of infected patients, blinding of all caregivers was not possible. All patients and outcome assessor were blinded to the arms of study.

Deputy of the vice chancellor of research and technology in Shahid Beheshti University of Medical Sciences provided all supports.

All patients were randomly assigned to each arm of the study via permuted block randomization (each block-sized for three or six patients) in order to minimize allocation bias in each study group. The sequence of the randomization was generated via “randomizeR” package using R project for statistics computing version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). All randomization codes for individuals enrolled to the study, were sealed in unrecognizable opaque envelopes by the responsible statistician for randomization. The investigator (IAD, MMR, and FH) enrolled the patients and only then open envelopes to assign patients to the different treatment groups. This method of allocation concealment leads to minimizing selection and confounding biases.

The Ethics in Medical Research Committee of the Shahid Beheshti University of Medical Sciences had been confirmed the study. Signed informed consents were obtained from all participants or their legally authorized representatives. The trial was carried out under the declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) guidelines for the conduct of clinical trials on human participants. The trial has been registered with ClinicalTrials.gov, NCT04521400 and the full protocol is freely available on the BMC Trials(12).

Patients

In this randomized controlled trial, patients were assigned to the intervention group or the control group. The inclusion criteria were as follow: age ≥ 18 years, oxygen saturation (SPO2) ≤ 93% or respiratory rate ≥ 24, presence of at least one of following manifestations on admission: Cough, shortness of breath, nasal congestion/ discharge, myalgia/arthralgia, radiation contactless body temperature ≥ 37.8, diarrhea/vomiting and headache or fatigue. The patients’ symptoms must be in acute phase (≤ 14 days).

Exclusion criteria were refusal to participate, receiving drugs with interactions with lopinavir/ritonavir or Interferon-β 1a, a fivefold rise in serum AST/ALT relative to upper limit of normal laboratory results, pregnant or lactating women, history of alcohol or drug addiction in the past 5 years and intubated less than one hour after admission to the hospital.

Clinical And Laboratory Monitoring

For assuring safety, daily monitoring for adverse effects (AEs) and treatment-related AEs, vital signs (pulse rate, respiratory frequency, body temperature, and blood pressure), spo2, Glasgow Coma Scale (GCS) and laboratory tests was performed. Time, severity, symptoms of adverse effects and their relation with aforementioned drugs on a daily basis were recorded.

Before enrollment, nasopharyngeal swab samples were obtained from each patient. The samples were tested using RT-PCR kits including Liferiver (W-RR-0479-02, China) for E, N, and Rdrp genes. Patients' data were recorded on paper checklists and Hospital Information System (HIS) by FH and MMR. The recorded data were entered into a pre-designed EXCEL sheet and later confirmed by a third investigator (IAD).

Outcome Measures

Primary outcome measure was TTCI defined as the time from enrollment to discharge or decline of two steps on the seven-step ordinal scale. Beigel and colleagues in a posthoc analysis of an influenza study introduced a six-step ordinal scale. WHO R&D Blueprint Team (Accessed May 15, 2020, at https://www.who.int/teams/blueprint/covid-19) have recommended a nine-step ordinal scale for COVID-19. In the current study the utilized seven-step ordinal scale consists of the subsequent categories: (I) Not hospitalized, and has no activity limitations; (II) Not hospitalized, but has activity limitations; (III) Hospitalized, but does not need any supplemental oxygen; (IV) Hospitalized, and needs supplemental oxygen; (V) Hospitalized, and needs either High-Flow Nasal Cannula (HFNC) or non-invasive ventilation; (VI) Hospitalized, and needs invasive ventilation; and (VII) Dead.

Secondary outcomes include mortality from the date of randomization until day 21, by which all of the patients will have at least one of the following outcomes: 1) A decline of two steps on the seven-step ordinal scale, 2) Hospital discharge or 3) Death. SpO2 improvement defined as the difference between the last and the first recorded measurement during the hospitalization, using pulse oximetry; length of stay in the hospital until the date of discharge from hospital or death from any cause, whichever came first; the incidence of new mechanical ventilation uses from the date of randomization until day 21. Follow-ups of discharged patients were done by utilizing telemedicine visits, online, or over the telephone.

Statistical Analysis:

Total sample size was calculated according to the Latouche and colleagues approach for estimating sample size in survival analyses with 90% power, alpha = 0.05, Hazard Ratio (HR) of 2.0 (as the ratio of the hazard rates of TTCI) and assuming that 60% of patients would reach the primary outcome. The calculations were carried out using Package 'powerSurvEpi' in R and accounted for a dropout rate of 10%. According to above-mentioned assumptions, 168 patients should have been recruited for this trial. Patients who failed to reach the primary endpoint (TTCI) or died prior to day 21 were regarded as right-censored in analysis.

Kaplan–Meier (compared with a log-rank test) was used to analyze the TTCI. Cox proportional-hazards model was also applied to calculate the HRs with 95% Confidence Intervals (CIs). All the participants who had undergone randomization were included in Intention-To-Treat (ITT) analysis (Fig. 1).

For categorical variables frequencies and percentage were employed. For distributed continuous variables Mean (SD) and for none-normally distributed variables, median (interquartile range) were used, respectively. Categorical variables were analyzed using chi-squared or the Fisher's exact test (when the expected frequency was less than 5 in one or more cells). Continuous variables were evaluated using T-test (for normally distributed) and Mann-Whitney U test (for non-normally distributed). A p-value of < 0.05 was considered to be statistically significant. All of the carried-out tests were two-tailed. R software version 3.6.1 was used to perform the statistical analyses.

Results

Patients:

Of 410 patients with positive RT-PCR and/or chest CT scan, 168 patients with moderate to severe COVID-19 were recruited in the trial. Control group is comprised of 85 patients treated with low-dose IFN-β 1a and 83 patients were included in intervention group (treated with high-dose IFN-β 1a). The flowchart for the study was depicted in Fig. 1. Mean ± SD for participants was 59.9 ± 16.5. Percentages of male and female participants were 61.9% and 38.1%, respectively. Demographic and clinical baseline information for control and intervention group were outlined in Table 1. Majority of clinical factors failed to reach a significant difference between two studied groups at baseline. However, some risk factors including spo2, ferritin, D-dimer were significantly different between groups at baseline ($p < 0.05$). In intervention group, the frequency of cases with D-dimer > 1000 ng/nl and spo2 < 90% were significantly higher than those in control group (for D-dimer 19.3% vs 3.1%; for Spo2 94% vs 81.2%).

Table 1
Characteristics of the Patients at Baseline*

Characteristic	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
Age (year)	59.8 (16.5)	59.6 (16.3)	60.1 (16.8)	0.85
Male sex — no. (%)	104 (61.9%)	56 (65.9%)	48 (57.8%)	0.28
BMI (kg/m2)	27.5 (5.8)	28.5 (6.1)	26.8 (5.5)	0.08
Duration of symptoms before presentation < 7 days	137 (81.5%)	68 (80.0%)	69 (83.1%)	0.60
Past medical history				
Diabetes	45 (26.8%)	25 (29.4%)	20 (24.1%)	0.44
Hypertension	62 (37.3%)	25 (30.1%)	37 (44.6%)	0.054
Ischemic Heart Disease	31 (18.7%)	18 (21.7%)	13 (15.7%)	0.32
Congestive heart failure	19 (11.4%)	13 (15.7%)	6 (7.2%)	0.09
Cerebrovascular accident (CVA)	9 (5.4%)	2 (2.4%)	7 (8.4%)	0.09
Coronary Heart Disease	25 (14.9%)	14 (16.5%)	11(13.3%)	0.56
Chronic Kidney Disease	14 (8.3%)			
Malignancy	1 (0.6%)	1 (1.2%)	0 (0.0%)	0.32
Chronic Obstructive Pulmonary Disease	2 (1.2%)	1 (1.2%)	1 (1.2%)	1.000
Asthma	3 (1.8%)	0 (0.0%)	3 (3.6%)	0.08
Paramedical history				
Anti-viral drug	8 (4.7%)	3 (3.5%)	5 (6%)	0.48
Steroid	2 (1.1%)	0 (0.0%)	2 (2.4%)	0.15
ACE & ARB	44 (26.3%)	15 (17.9%)	29 (34.9%)	0.012
Risk factors for severe disease				
RF Respiratory Rate > 24/min — no· (%)	103 (61.3%)	39 (45.9%)	64 (77.1%)	< 0.001

The values shown are based on available data. Value for D.dimer was available for 64, values for CPK was available for 79 patients and values for DLH was available for 80 patients in low dose group. Values for Erythrocyte Sedimentation Rate was available for 60 patients in low dose group and 63 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

Characteristic	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
RF Oxygen Saturation (SpO2) (< 90%)	147 (87.5%)	69 (81.2%)	78 (94.0%)	0.012
RF D.dimer (> 1000 ng/ml)	18 (12.2%)	2 (3.1%)	16 (19.3%)	0.003
RF CPK (> twice upper limit of normal)	55 (34.2%)	28 (35.4%)	27 (32.9%)	0.74
RF CRP (> 100 mg/liter)	8 (4.8%)	8 (9.6%)	0 (0.0%)	0.004
RF LDH (> 245 U/liter)	148 (95.5%)	70 (95.9%)	78 (95.1%)	0.82
RF Lymphcount (0.8×10^{-9}/liter)	65 (39.2%)	28 (33.7%)	37 (44.6%)	0.15
RF Ferritin (> 300 µg/L)	137 (87.3%)	61 (82.4%)	76 (93.8%)	0.027
Heart Rate (> 125/min)	69 (41.1%)	27 (31.8%)	42 (50.6%)	0.013
Respiratory factors				
Oxygen Saturation (SpO2) — median (IQR)	84.0 (76.0–88.0)	86.0 (80.0–88.0)	83.0 (70.0–87.0)	0.006
Venous PCO2, median (IQR)	38.0 (31.3–45.0)	38.0 (30.2–46.4)	38.0 (32.9–44.0)	0.95
Venous HCO3, median (IQR)	24.9 (22.1–28.0)	24.0 (22.1–28.0)	25.0 (22.0–28.0)	0.88
White Blood Cell count ($\times 10^{-9}$/liter)				
$< 4 \times 10^{-9}$/liter — no. (%)	11 (6.7%)	6 (7.4%)	5 (6.0%)	0.83
$4–10 \times 10^{-9}$/liter — no. (%)	97 (59.1%)	49 (60.5%)	48 (57.8%)	
$> 10 \times 10^{-9}$/liter — no. (%)	56 (34.1%)	23 (32.1%)	30 (36.1%)	
Lymphocyte count ($\times 10^{-9}$/liter) —median (IQR)	0.97 (0.60–1.49)	0.92 (0.60–1.52)	0.97 (0.59–1.42)	0.72
$\geq 1.0 \times 10^{-9}$/liter — no. (%)	76 (46.6%)	37 (45.7%)	39 (47.6%)	0.81

The values shown are based on available data. Value for D.dimer was available for 64, values for CPK was available for 79 patients and values for DLH was available for 80 patients in low dose group. Values for Erythrocyte Sedimentation Rate was available for 60 patients in low dose group and 63 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

Characteristic	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
< 1.0×10^{-9}/liter — no. (%)	87 (53.4%)	44 (54.3%)	43 (52.4%)	
Neutrophil count (× 10^{-9}/liter) — median (IQR)	6.75 (4.21–9.53)	6.67 (4.02–9.33)	6.75 (4.29–10.44)	0.40
< 1.5×10^{-9}/liter — no. (%)	1 (0.6%)	1 (1.3%)	0 (0.0%)	0.61
1.5-8 × 10^{-9}/liter — no. (%)	97 (63.0%)	49 (62.8%)	48 (63.2%)	
> 8×10^{-9}/liter — no. (%)	56 (36.4%)	28 (36.8%)	28 (36.8%)	
Platelet count (× 10^{-9}/liter) — median (IQR)	198.0 (148.5–272.0)	180.5 (134.5-230.5)	222 (153–315)	0.88
≥ 100×10^{-9}/liter — no. (%)	151 (91.5%)	76 (92.7%)	75 (90.4%)	0.59
< 100×10^{-9}/liter — no. (%)	14 (8.5%)	6 (7.3%)	8 (9.6%)	
Serum Creatinine (µmol/liter) — median (IQR)	110 (100–140)	110 (100–140)	110 (100–140)	0.99
≤ 133 µmol/liter — no. (%)	122 (73.1%)	61 (72.6%)	61 (73.5%)	0.89
> 133 µmol/liter — no. (%)	45 (26.9%)	23 (26.5%)	22 (26.5%)	
Aspartate Aminotransferase (AST) (U/liter) — median (IQR)	55 (41.7–80.5)	54 (40–80)	55 (42–82)	0.41
≤ 40 U/liter — no. (%)	37 (22.3%)	21 (25.3%)	16 (19.3%)	0.35
> 40 U/liter — no. (%)	129 (77.7%)	62 (74.7%)	67 (80.7%)	
Alanine Aminotransferase (ALT) (U/liter) — median (IQR)	44.5 (25.2–69.5)	42 (25–54)	49 (25-95.5)	0.07
≤ 50 U/liter — no. (%)	98 (59.8%)	56 (67.5%)	42 (51.9%)	0.04
> 50 U/liter — no. (%)	66 (40.2%)	27 (32.5%)	39 (48.1%)	
Lactate Dehydrogenase (LDH) (U/liter) — median (IQR)	583 (416–779)	563 (418-765.5)	656 (411.5–784)	0.42
≤ 245 U/liter — no. (%)	7 (4.5%)	3 (4.1%)	4 (4.9%)	0.82

The values shown are based on available data. Value for D.dimer was available for 64, values for CPK was available for 79 patients and values for DLH was available for 80 patients in low dose group. Values for Erythrocyte Sedimentation Rate was available for 60 patients in low dose group and 63 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

Characteristic	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
> 245 U/liter — no. (%)	148 (95.5%)	70 (95.9%)	78 (95.1%)	
Blood Urea Nitrogen (BUN) — median (IQR)	42 (30.7–61)	45 (33-60.7)	39.5 (25.7–64)	0.22
C-Reactive Protein (CRP) — median (IQR)	48 (28.9–63.7)	52 (33.5–73.6)	45 (26.4–55.4)	0.013
CRP < 6 — no. (%)	6 (4.8%)	3 (5.3%)	3 (4.3%)	0.81
CRP > 6 — no. (%)	120 (95.2%)	54 (94.7%)	66 (95.7%)	
Erythrocyte Sedimentation Rate (ESR) — median (IQR)	50 (38–65)	47.5 (28.2–60)	53 (44–66)	0.09

The values shown are based on available data. Value for D.dimer was available for 64, values for CPK was available for 79 patients and values for DLH was available for 80 patients in low dose group. Values for Erythrocyte Sedimentation Rate was available for 60 patients in low dose group and 63 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

Primary Outcome:

Median TTIC for cases treated with low dose of IFN-β1a was shorter than that for cases treated with high dose IFN-β1a (6 vs10 days; P = 0.018) (Table 2). In the Cox regression model, HR was 1.56 (95% CI: 1.05–2.30, P-value = 0.026).

Table 2
Outcomes in the Intention-to-Treat Population*

	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
Mortality at day 21 — no. (%)	65 (38.7%)	31 (36.5%)	34 (41.0%)	0.55
Hospital stay — median no. of days (IQR)	8 (6–9)	6 (5–7)	10(8–12)	0.018
Respiratory factors				
Oxygen Saturation (Worst) (SpO2) — median (IQR)	78.0 (62.0–84.0)	80.5 (64.0-86.2)	75.0 (60.0–82.0)	0.017
Oxygen Saturation (Discharge) (SpO2) — median (IQR)	91.0 (88.7–93.0)	92.0 (89.0-94.5)	90.0 (88.0–92.0)	0.017
Venus Pco2 (Discharge)— median (IQR)	41.0 (38.0-45.4)	41.0 (39.0–44.0)	41.0 (36.8–50.0)	0.61
Venus Hco3(Discharge)— median (IQR)	25.8 (24.1–27.0)	25.0 (25.0–27.0)	25.8 (22.1–27.0)	0.48
White Blood Cell count ($\times 10^{-9}$/liter)				
$< 4 \times 10^{-9}$/liter — no. (%)	22 (13.4%)	14 (16.9%)	8 (9.9%)	0.22
$4–10 \times 10^{-9}$/liter — no. (%)	90 (54.9%)	47 (56.6%)	43 (53.1%)	
$> 10 \times 10^{-9}$/liter — no. (%)	52 (31.7%)	22 (26.5%)	30 (37.0%)	
Lymphocyte count ($\times 10^{-9}$/liter) — median (IQR)	0.88 (0.54–1.51)	0.97 (0.58–1.42)	0.89 (0.56–1.48)	0.83
$\geq 1.0 \times 10^{-9}$/liter — no. (%)	66 (41.5%)	36 (43.9%)	30 (39.0%)	0.53
$< 1.0 \times 10^{-9}$/liter — no. (%)	93 (58.5%)	46 (56.1%)	47 (61.0%)	
Platelet count ($\times 10^{-9}$/liter) — median (IQR)	207 (146–279)	208 (160-275.2)	198 (142–280)	0.39

*Values for HCT was available for 76 patients in low dose group and 79 patients in high dose group. Values for Erythrocyte Sedimentation Rate was available for 62 patients in low dose group and 64 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

	Total (N = 168)	Low-dose (N = 85)	High-dose (N = 83)	P-value
≥ 100 × 10^{-9}/liter — no. (%)	152 (93.3%)	77 (93.95%)	75 (92.6%)	0.74
< 100 × 10^{-9}/liter — no. (%)	11 (6.7%)	5 (6.1%)	6 (7.4%)	
Neutrophil count (× 10^{-9}/liter) — median (IQR)	6.22 (4.07–9.25)	5.50 (3.61–8.32)	6.85 (4.65–9.74)	0.10
< 1.5 × 10^{-9}/liter — no. (%)	5 (3.3%)	1 (1.3%)	4 (5.4%)	0.19
1.5-8 × 10^{-9}/liter — no. (%)	95 (62.5%)	53 (67.9%)	42 (56.8%)	
> 8 × 10^{-9}/liter — no. (%)	52 (34.2%)	24 (30.8%)	28 (37.8%)	
C-Reactive Protein (CRP) — median (IQR)	55 (41.8.-73)	66.1 (45–83)	51.9 (36.1–62.7)	0.004
CRP < 6 — no. (%)	4 (2.9%)	2 (2.8%)	2 (2.9%)	0.96
CRP > 6 — no. (%)	135 (97.1%)	69 (97.2%)	66 (97.1%)	
Erythrocyte Sedimentation Rate (ESR) — median (IQR)	53.5 (40–68)	50 (30.5–69.2)	59 (44.2–68.0)	0.17

*Values for HCT was available for 76 patients in low dose group and 79 patients in high dose group. Values for Erythrocyte Sedimentation Rate was available for 62 patients in low dose group and 64 patients in high dose group. Quantitative measures were compared using the Mann–Whitney U test or (if normally distributed) T-test. Categorical variables were compared using the Chi-Square test or Fisher exact test.

Due to differences between some clinical factors between intervention and control group at baseline, we; therefore, performed an adjusted analysis by including spo2, D-dimer and CRP in Cox regression model. The model failed to reach a significant difference between two groups. The adjusted HR was 1.37 (95% CI: 0.88–2.12, P-value = 0.16).

Secondary Outcome:

In this study, of 168 participants, total mortality number was 65. The mortality rates in intervention and control group were 34 (41%) and 31 (36.5%), respectively. No significant difference was found in terms of mortality rate between two groups. Length of stay in hospital for patients treated with high-dose was longer than that in control group (Table2). Intervention group exhibited lower oxygen saturation and respiratory rate compared with control group (P = 0.017). Rest of clinical factors were outlined in Table 2.

Safety:

Table 3 outlines adverse events in the safety population. In the intervention group, the prevalence of skin rash was significantly higher compared to control group (13.3% vs .35%). On the other hand, in the patients treated with low-dose, the number of cases with leukopenia, hypo-albuminemia and severe anemia were significantly higher relative to the intervention group (Table 3).

Table 3
Adverse Events in the Safety Population. *

Event	Low-dose (N = 85)	High-dose (N = 83)	
Adverse Event			**P Value**
Nausea	16 (19.0%)	17 (20.5%)	0.82
Vomiting	5 (6.0%)	4 (4.8%)	0.73
Diarrhea	10 (11.8%)	9 (10.8%)	0.85
Abdominal pain	14 (16.5%)	10 (12.0%)	0.41
Rash	3 (3.5%)	11 (13.3%)	0.023
Raised LFT	29 (34.1%)	29 (34.9%)	0.91
Hyperbilirubinaemia	19 (22.6%)	20 (24.1%)	0.82
Increased Creatinine	22 (25.9%)	21 (25.3%)	0.93
Leukopenia	22 (26.2%)	7 (8.4%)	0.002
Anemia	28 (33.3%)	26 (31.3%)	0.78
Hypo.Albuminemia	9 (11.3%)	2 (2.4%)	0.024
Rised CPK	14 (16.7%)	12 (14.6%)	0.72
Lymphopenia	39 (45.9%)	36 (43.9%)	0.80
Serious Adverse Event			
Acute Respiratory Distress Syndrome (ARDS)	27 (31.8%)	33 (39.8%)	0.28
Acute Kidney Failure (AKI)	15 (17.6%)	11 (13.3%)	0.43
Secondary Infection	5 (5.9%)	8 (9.6%)	0.36
Shock	21 (24.7%)	15 (18.1%)	0.29
Severe Anemia	17 (20.0%)	6 (7.3%)	0.02
Acute gastritis	1 (1.2%)	0 (0.0%)	0.32
Lower GI bleeding	1 (1.2%)	3 (3.6%)	0.30
Sepsis	15 (17.6%)	14 (16.9%)	0.89
Pneumothorax	0 (0.0%)	0 (0.0%)	…

*Adverse events that occurred in more than one patient after randomization through day 21 are shown. Some patients had more than one adverse event.

Discussion

In the current study, our major finding was that the high-dose IFN-β 1a administration not only was not associated with lower mortality but also increased the length of stay in hospital as opposed to low-dose IFN-β 1a administration. Based on clinical records, better outcome (proper oxygen saturation) was observed in patients treated with lower dose of interferon.

Interferons have different types with potent immunomodulatory and antiviral effects. IFN-β as a type 1 interferon has been used to treat immune medicated disorders due to its immune regulatory properties. In the course of viral infection, interferon expression is associated with boosted host defense against the viral phase of the infection (13). Absence or lack of interferon production or presence of autoantibodies against interferons leads to easay spread of virus. Therefore, interferons have detrimental roles in minimizing the extent of severe infections. In a recent study, it has been showed that defects in interferon genes as well as core genes responsible for production of molecules in interferon amplification pathway, was associated with severe COVID-19. Therefore, type one INF administration in cases with severe COVID-19 was recommended by the investigator (14).

Antiviral effects of various agents against coronaviruses have been studied. Of all types of INFs, the most significant antiviral effect against MERS-CoV has been mentioned for INF β subgroup (15, 16). Among various types of INFs, IFN-β 1a has been associated with clinical efficacy compared to IFN-β 1b in the treatment of COVID-19 (17).

In our study, results revealed that the mortality rate was not statistically different between patients treated with high- and low-dose IFN-β 1a. On the other hand, prolonged hospital stays and improper oxygenation status was significantly higher in patients treated with high-dose of IFN-β 1a. It is of note to consider that intervention group had lower oxygen saturation at baseline. In addition, clinical and laboratory parameters including respiratory rate, heart rate, ferritin, D-dimer were significantly different between two studied groups at baseline. An adjusted analysis conducted by including spo2, D-dimer, and CRP in a Cox regression model, exhibited that there was no statistically significant difference between two groups.

Differences in length of hospital stay between two groups was another impressive finding. It is obvious that prolonged length of hospitalization is associated with increased risk of complication and secondary infections. Moreover, it definitely places financial burden on either patient and healthcare systems. Furthermore, we should consider differences in baseline characteristics of our patients which demonstrate more severe disease at baseline. A more severe course could be associated with a longer duration of hospitalization.

In previous studies, some conflicting data have been reported. In a randomized placebo-controlled study on adult patients with moderate to severe COVID-19 IFN-β 1a administration was not associated with clinical benefits (18). In the respect study, IFN-β 1a was administered 10 μg once daily by intravenous route for 6 days and there was difference between IFN-β 1a dosage in our study and theirs. In another open-label randomized trial in Hong Kong, early (≤ 7days) administration of IFN-β 1a by the dose of

8 million international units in combination with antiviral therapy was associated with reduction in duration of viral shedding, symptoms alleviation and reduced hospital stay (19). This study; however, enrolled patients with mild to moderate disease and it was not designed as a placebo-controlled study. Interim results from the largest randomized control trial on COVID-19, coordinated by the World Health Organization therapeutics indicated that IFN-β 1a have little or no effect on 28-day mortality or in-hospital course of COVID-19 among hospitalized patients (20).

High-dose IFN-β 1a (12 million international units) was considered for treatment of other conditions such as immune-mediated diseases (21). Number of clinical trials assessing the benefits of high dose interferon beta 1a on patients with COVID-19 is scarce. In a study, it has been shown that addition of high dose IFN-β 1a to antiviral agents (lopinavir/ritonavir and hydroxychloroquine) was associated only with symptoms alleviation but the study had been conducted as a non-controlled clinical trial (22). In our study, although majority of patient had severe COVID-19 infection, the mortality rate was lower compared to other studies(23, 24). Therefore, it can be assumed that IFN-β 1a could be effective in COVID-19 treatment.

By administering higher doses of interferon, we expect to experience some adverse reactions dominantly. For instance, Higher doses can lead to hepatic toxicity. Therefore, In this cases dose reduction should be considered (25). Administration of lopinavir/ritonavir also was associated with hepatic toxicity and liver enzymes elevation (26). Hence, we were uncertain that interferon administration was the cause of liver enzyme elevation. Moreover, we could not observe any difference in this regard between two groups.

In our study, skin rash was more prevalent in patients treated with high-doses of interferon. Some serious hematologic adverse effects were observed more frequently in patients treated with low-doses of interferon. Hematologic side effects and bone marrow suppression are well known adverse effects associated with interferon use (27). It has been proven that these side effects are categories as late adverse effects and are associated with the duration of exposure. However, in our study, leukopenia was more prevalent in control group. Owing to various other clinical factors which should be taken under consideration, we should not consider IFN-β 1a administration as the only effective variable on hematologic findings in our study.

Limitations

First and the most important limitation of the study is baseline characteristic variation. Although the study was designed as a prospective randomized clinical trial, because of limited sample size, different dispersion was observed in some baseline factors between patients in two groups. Second, it was not possible to analyze arterial blood gas for some patients because of technical procedures limitation and trained staff limitation.

Conclusions

In this trial involving hospitalized patients with moderate to severe COVID-19, we did not find a significant difference in mortality rate between groups treated with high- and low dose. The median TTCI was better in the control group compared with the intervention group.

Declarations

Ethics approval: The trial was confirmed by the Ethics in Medical Research Committee of the Shahid Beheshti University of Medical Sciences. signed informed consents were obtained from all the participants or their legally authorized representatives. This trial has been registered as ClinicalTrials.gov, NCT04521400.

Consent for publication: Not applicable

Availability of data and materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions: All authors contributed to conception and design of study; MMR, FH, IAD, contributed to the acquisition of data; MMR, FH, IAD and MAP contributed to the analysis of data; all authors contributed to the drafting of the article and/or critical revision; and all authors contributed to the final approval of manuscript.

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Figures

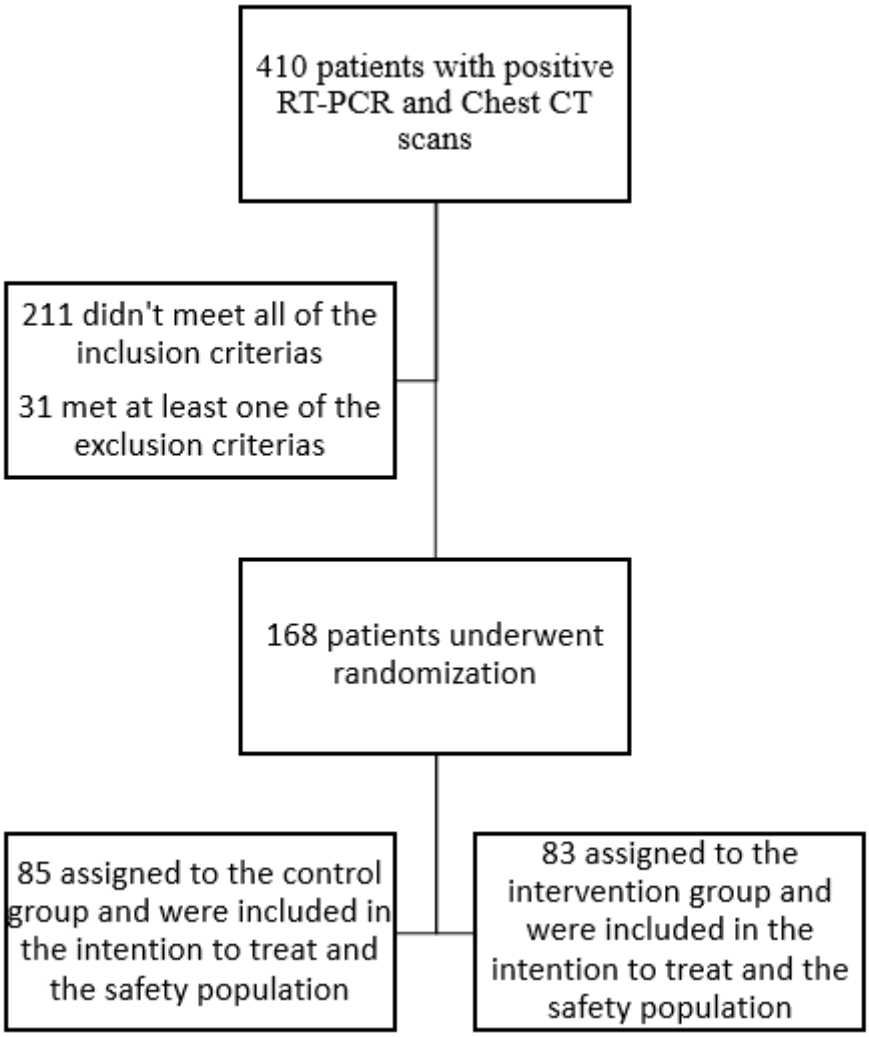

Figure 1

Trial Flow Diagram

Supplementary Files

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