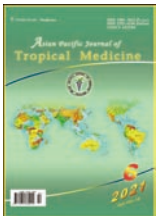

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Ivermectin as an adjunct treatment for hospitalized adult COVID-19 patients: A randomized multi-center clinical trial

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ABSTRACT

Objective: To evaluate different doses of ivermectin in adult patients with mild COVID-19 and to evaluate the effect of ivermectin on mortality and clinical consequences.

Methods: A randomized, double-blind, placebo-controlled, multicenter clinical trial was performed at five hospitals. A total of 180 mild hospitalized patients with COVID-19 confirmed by PCR or chest image tests were enrolled and allocated to six arms including hydroxychloroquine 200 mg twice per day, placebo plus hydroxychloroquine 200 mg twice per day, single dose ivermectin (200 μg/kg), three low interval doses of ivermectin (200, 200, 200 μg/kg), single dose ivermectin (400 μg/kg), and three high interval doses of ivermectin (400, 200, 200 μg/kg). The primary endpoint of this trial was all-cause of mortality or clinical recovery. The radiographic findings, hospitalization and low O_2 saturation duration, and hematological variables of blood samples were analyzed.

Results: A total of 16.7% (5/30) and 20.0% (6/30) patients died in arms treated with hydroxychloroquine 200 mg twice per day and placebo plus hydroxychloroquine 200 mg twice per day, respectively, and a reduction in mortality rate in patients receiving ivermectin treatment to 0%, 10%, 0% and 3.3% for arms 1-4 were observed. Risk of mortality was also decreased about 15% in the ivermectin treated arms.

Conclusions: Ivermectin as an adjunct reduces the rate of mortality, time of low O_2 saturation, and duration of hospitalization in adult COVID-19 patients. The improvement of other clinical parameters shows that ivermectin, with a wide margin of safety, had a high therapeutic effect on COVID-19.

KEYWORDS: SARS-COV-2; Ivermectin; Randomized controlled trial; Dose-respond relationship

1. Introduction

The COVID-19 disease has become a pandemic after the WHO declaration in March 2020. This disease has created a difficult condition around the world, and hence there is an important and urgent need to find proper treatments for an effective cure, decrease the virus carriage duration, and thus limit its transmission in society[1-3]. So far, different drugs such as hydroxychloroquine, azithromycin, remdesivir, oseltamivir, lopinavir, and ritonavir have been used against COVID-19[4-10]. However, among the candidate treatments, only remdesivir have been tested in large

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comparative studies. Although, some studies have shown that remdesivir have no clear influence on COVID-19 disease and it is associated with some adverse effects[8,9]. Ivermectin is an inexpensive FDA-approved anti-parasitic drug, known to have a wide-spectrum antiviral activity against a variety of viruses in *in–vitro* conditions. It inhibits the proliferation of COVID-19 in cell culture as well[11–13]. Moreover, ivermectin shows antiviral activity against some RNA viruses including Zika, Dengue, Yellow Fever, West Nile, Hendra, Newcastle, Venezuelan equine encephalitis, Chikungunya, Semliki Forest, Sindbis, Avian influenza A, porcine reproductive and respiratory syndrome, and HIV-1. Furthermore, there are some studies showing the antiviral effects of ivermectin against DNA viruses such as Equine herpes type 1, BK polyomavirus, pseudorabies, porcine circovirus 2, and bovine herpesvirus 1[14]. Ivermectin may also act on the SARS-CoV-2 virus through three proposed mechanisms that resulted in reducing the load of the virus. These mechanisms includes: inhibition of nuclear import of host[15], interfering with the attachment of the spike to the human cell membrane[16,17], and inhibition of helicase activity[18]. As illustrated in Figure 1, the main *in vitro* proposed mechanism of activity of ivermectin hinders the viral load through inhibition of the interaction of importin and β 1 proteins. Several clinical trial studies have reported the effect of ivermectin on the treatment of COVID-19 patients[19,20]. The survey of studies on COVID-19 in recent months and ongoing clinical trials in various communities show that ivermectin as a low-cost, highly available drug is increasingly considered as a treatment option specially in low-income countries. Given the high number of patients with persistent symptoms and the favorable clinical response to receiving a specific treatment, we designed a strategy of adding ivermectin to treatment regimen at different doses, to investigate appropriate dose and its possible treatment efficacy on COVID-19 patients.

2. Subjects and methods

2.1. Study design

A randomized, double-blind, placebo-controlled, multicenter study was carried out to examine the effectiveness of oral ivermectin in hospitalized adults (age>18 years) with COVID-19. In Iran, we restricted our target population to Qazvin and Khuzestan given the trajectory of the virus' spread in these provinces. The trial was conducted at five hospitals (Velayat, Bu Ali, Taleghani, Razi, and Sina) in two provinces.

2.2. Ethics statement and informed consent

Ethical approval was in accordance with the ethical standards of the Helsinki Declaration (1964, amendment of 2008). The study protocol was approved by the medical ethics committee of Qazvin University of Medical Sciences (registration ID IR.QUMS.REC.1399.017). A participant information sheet containing the particulars of the study was provided to all the patients and informed consent was taken from all the patients. Participant information sheet and informed consent were available in Persian. The details of the reports were kept completely confidential. This trial was registered with the Iranian Registry of Clinical Trials website (registration ID: IRCT20200408046987N1).

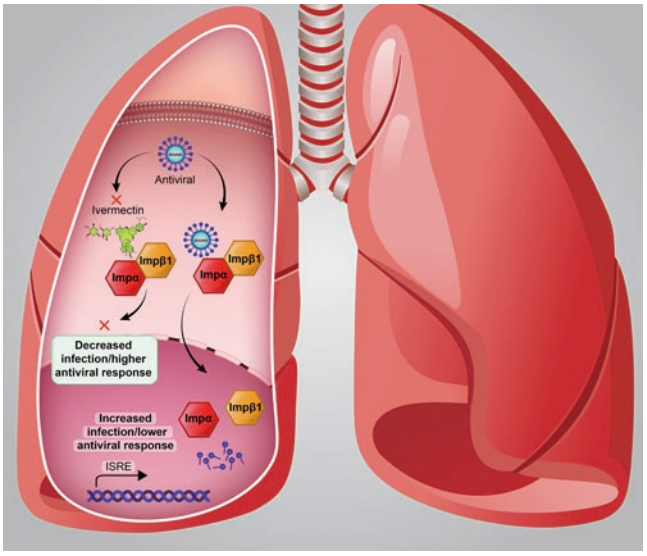

Figure 1. Mechanism of ivermectin inhibition on coronavirus.

2.3. Patient selection

Eligible patients with COVID-19 who met the following criteria were admitted: (1) age>18 years; (2) signed the informed consent; (3) clinical symptoms of suggestive of COVID-19 pneumonia: cough (with or without sputum), fever, pleuritic chest pain or dyspnea; (4) mild to severe COVID-19 disease confirmed by chest computed tomography (CT) scan findings compatible with COVID-19 or positive real-time reverse transcription polymerase chain reaction (RT-PCR). Exclusion criteria included children (Children have much lower rates of severe COVID-19), presence of severe immunosuppression (*e.g.*, use of immune-suppressants and HIV positive), pregnant women (risk to fetus/infant and low numbers of potential participants of this profile would limit investigators' ability to understand efficacy and safety in pregnant or nursing patients), a known allergic reaction to the intervention drugs, chronic kidney disease, malignancy, sever COVID-19 patients and indications that the patients were unable and/or unlikely to comprehend and/or follow the protocol. The patients were enrolled in the study after writing and signing the inform consent letter by themselves or a legal representative if they were unwell and unable to do it.

2.4. Sample size

Considering the designing of this study based on drug-dose and outcome scale as a qualitative trait, comparison of the recovery rate and two-sided response test was used to calculate the sample size as follows:

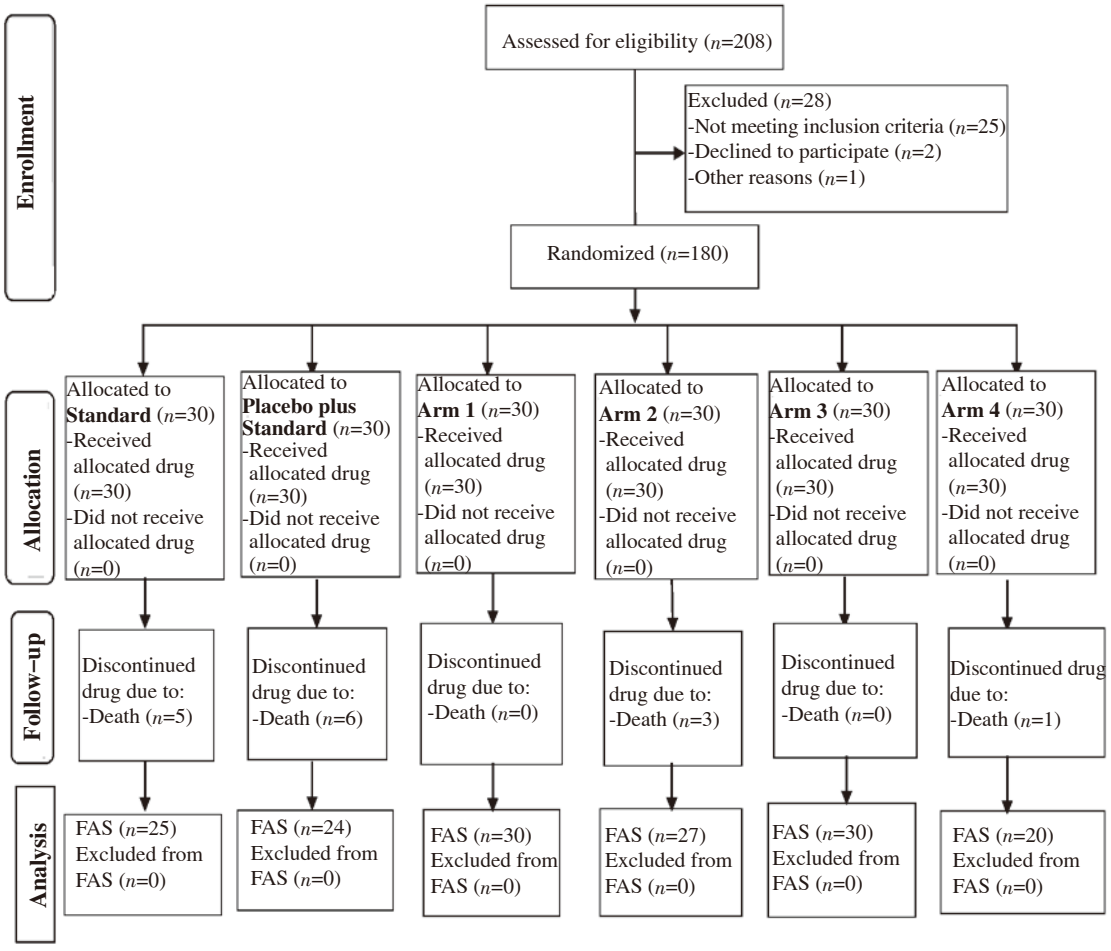

Figure 2. Diagram of participants evaluated and enrolled in this study.

$$N = Z_{1-\alpha}\left(\sqrt{\sum_{i}^{k}\frac{ci^2}{fi}\bar{p}(1-\bar{p})} + Z_{1-\beta}\sqrt{\sum_{i}^{k}\frac{ci^2}{fi}\bar{p}i(1-\bar{p}i)}\right)^2$$

Convenient sample size of 530 patients was examined to obtain 180 participants. Finally, 30 patients entered each arm.

2.5. Randomization and masking

Eligible patients were randomly allocated to either the standard, placebo and the ivermectin arms. The transposed block randomization sequence, including stratification, was prepared by a statistician not involved in the trial using Random Allocation Software. The investigator and patients were masked.

2.6. Interventions

The participants of study were allocated to six arms including hydroxychloroquine 200 mg twice per day based on Iran Health Ministry, placebo plus hydroxychloroquine 200 mg twice per day, single dose ivermectin (200 μg/kg), three low interval doses of ivermectin (200, 200, 200 μg/kg), single dose ivermectin (400 μg/kg), and three high interval doses of ivermectin (400, 200, 200 μg/kg). All patients were treated according to "Iranian Guideline of Hospitalized COVID-19 Patients' Management (Version 5)". This comprised oral hydroxychloroquine 200 mg twice per day as standard regimen and a heparin prophylaxis in combination with supplemental oxygen. Tablet of ivermectin (14 mg) and placebo were formulated in Alborz Darou pharmaceutical Co., Qazvin, Iran.

2.7. Outcome measures

The primary endpoint of this trial was all-cause of mortality or clinical recovery. Clinical recovery was defined as normal temperature, respiratory rate, and oxygen saturation (>94%) without oxygen therapy sustained for 24 h. The patients would be discharged if this trend continued. During the process criteria discharge changed. Initially, patients with two successive negative nasopharyngeal samples based on PCR assay (CT value 35) were separated. The secondary outcomes were the time until discharge from the hospital. Oxygen saturation testing and blood sampling absolute lymphocyte count (ALC), C-reactive protein (CRP), white blood cells (WBC), thrombocyte count (PLT), erythrocyte sedimentation rate (ESR), blood urea nitrogen (BUN),

and creatinine (Cr) were examined on day zero and day five. Assessment of gas exchange requires knowledge of fractional inspired oxygen tension (FiO_2); unless the patient is breathing room air. Hence, all peripheral capillary oxygen saturation was measured in breathing room air at rest.

2.8. Statistical methods

The SPSS version 20 (SPSS®, Armonk, NY, USA) software was used for statistical analyses. Kolmogorov-Smirnov test was used to evaluate normality of numerical variables. Differences in values of quantitative variable on days zero and five (△0/5) were calculated to check recovery process of patients between groups. Quantitative variables were expressed as means ± SD (standard deviations), or median (inter-quartile ranges, IQR), and qualitative variables as proportion. The comparison of the quantitative variables was accomplished with analysis of variance Kruskal-Wallis *H* test, *t*-tests or Mann-Whitney *U*-tests, and for comparison of the qualitative variables, the Pearson *Chi*-squared test was used. A *P*-value less than 0.05 was considered statistically significant. Analyses were based on non-missing data.

3. Results

A clinical trial was performed to determine the therapeutic dose and effect of ivermectin for COVID-19. A total of 180 patients with mild infection, with positive PCR or chest CT image examination, hospitalized at Qazvin and Khuzestan provinces hospitals (five hospitals) were selected. The start and end dates of patients recruitment were from 1st of June to 15th of July 2020. The study flowchart is shown in Figure 1. Demographics (sex, age), obesity, severity on CT, respiratory rate (RR/min), fever (℃), systolic and diastolic blood pressure (Bp, mmHg), pulmonary rate (PR /min) and oxygen saturation (%) of the patients are reported in Table 1. Comparison of the variables at the day one and five of admission (△0/5) showed that most patients in ivermectin arms have more favorable outcomes than patients in standard and placebo arms. As reported in Table 2, complete blood count (CBC) evaluation of the patients among the standard and placebo and ivermectin groups (arms 1 to 4) shows that ivermectin had a good effect on blood biomarkers and improved other clinical parameters such as ALC, CRP, PLT, ESR. The majority of patients had the desired outcomes and were discharged from the hospitals. The results of preclinical consequences in Figure 2 indicate a reduction in mortality rate in patients receiving ivermectin treatment to 0, 10%, 0 and 3.3% for arms 1-4, respectively. Compared to the standard and placebo plus standard arms, the reduction in mortality was 16.7% (5/30) and 20% (6/30), respectively. Moreover, the time of hospitalization and low O_2 saturating showed significant difference between groups. The lowest time of hospitalization and low O_2 saturation was significantly observed in two arms of 1 and 3 with single dose of 200 and 400 μg/kg dose ivermectin respectively, in comparison with the two control groups of S and S+P (Table 3). As mentioned, the single dose of the drug showed a good effect in the comparison between the groups and the primary goal of our study was achieved. In another analysis, Tachypnea Off (day), Fever Off (day), duration of low O_2 Sat and duration on hospital stay (day) were examined respectively. As shown in Table 3, the time of hospitalization and low O_2 saturating showed significant difference

Table 1. Demographic and baseline characteristics of the study population.

Factor	S	S+P	Arm 1	Arm 2	Arm 3	Arm 4	*H* or χ^2 values	*P* value
Sex, *n* (%)							17.380	0.912
Male	16 (53.3)	14 (46.7)	12 (40.0)	19 (63.3)	16 (53.3)	13 (43.3)		
Female	14 (46.7)	16 (53.3)	18 (60.0)	11 (36.7)	14 (46.7)	17 (56.7)		
Age, yr	55 (45, 70)	58 (45, 68)	61 (42, 68)	53 (42, 65)	54 (47, 60)	54 (46, 65)	1.889	0.958
BMI, kg/m^2	26.0 (24.4, 27.6)	25 .6 (23.9, 26.9)	26.1 (24.8, 28.0)	26.4 (25.5, 27.2)	27.7 (25.7, 32.6)	25.1 (23.9, 26.2)	15.641	**0.004**
Severity on CT , *n* (%)							2.381	0.527
Negative	0 (0)	0 (0)	0 (0)	2 (6.7)	0 (0)	0 (0)		
Mild	4 (13.3)	5 (16.7)	8 (26.7)	2 (6.7)	4 (13.3)	2 (6.7)		
Moderate	23 (76.7)	23 (76.7)	21 (70.0)	20 (66.7)	21 (70.0)	23 (76.7)		
Severe	3 (10.0)	2 (6.7)	1 (3.3)	6 (20.0)	5 (16.7)	5 (16.7)		
PCR positive, *n*(%)	18 (60.0)	14 (46.7)	23 (76.7)	23 (76.7)	29 (96.7)	21 (70.0)	13.840	0.421
CT, *n*(%)	12 (40.0)	16 (53.3)	7 (23.3)	7 (23.3)	1 (3.3)	9 (30.0)	11.320	0.527
RR, per min$^{\#}$	28 (25, 29)	28 (27, 34)	28 (26, 29)	30 (28, 34)	22 (20, 24)	29 (28, 30)	45.919	0.744
Fever, ℃$^{\#}$	36.8 (36.7, 37.0)	36.8 (36.8, 37.2)	36.8 (36.4, 37.1)	36.8 (36.7, 37.2)	36.5 (36.5, 36.8)	37.0 (36.0, 37.2)	8.311	0.875
Systolic BP, mmHg$^{\#}$	110 (110, 125)	112 (110, 135)	125 (110, 125)	110 (110 , 125)	120 (110, 120)	140 (120, 160)	6.789	0.443
Diastolic BP, mmHg$^{\#}$	80 (80, 80)	80 (80, 80)	80 (80, 80)	80 (80, 80)	75 (55, 80)	80 (70, 90)	22.489	0.400
PR per min$^{\#}$	80 (75, 85)	80 (72, 85)	78 (75, 85)	80 (78, 95)	85 (80, 93)	85 (78, 100)	7.643	0.174
O_2 Sat, %$^{\#}$	89 (85, 91)	88 (85, 90)	90 (88, 92)	88 (85, 90)	91 (87, 94)	89 (82, 90)	18.391	**0.002**

S: hydroxychloroquine 200 mg twice per day, P: Placebo + hydroxychloroquine 200 mg twice per day, Arm 1: Single dose ivermectin (200 μg/kg), Arm 2: three dose ivermectin (200 μg/kg), Arm 3: single dose ivermectin (400 μg/kg), Arm 4: three dose ivermectin (400, 200, 200 μg/kg); Kruskall-Wallis *H* test was applied for comparisons of median (Q1, Q3) $^{\#}$scores (*H* values); Pearson *Chi* square test was applied for comparisons of other values.

Table 2. Changes (△) from baseline (day 0) in hematological and biochemical parameters at day 5 post admission in different arms.

Variables	S	S+P	Arm 1	Arm 2	Arm 3	Arm 4
△WBC5/0, per μL	-300 (-2 500, 800)	-350 (-1 200, 900)	-200 (1 600, 300)	150 (-900, 1000)	0 (-1 000, 300)	650 (-300, 1800)
$P^{\&}$	-	0.512	<0.001*	0.730	0.008*	0.530
P^{*}	0.512	-	0.002*	0.167	0.250	0.827
△ALC5/0, per μL	78 (-310, 340)	58 (-360, 408)	357 (5, 625)	373 (-38, 565)	414 (89, 588)	316 (-180, 845)
$P^{\&}$	-	0.876	0.047*	0.032*	0.009*	0.046*
P^{*}	0.876	-	0.047*	0.033*	0.033*	0.009*
△PLT5/0, per μL	2.0 (-11, 27)	4.0 (-27, 28)	40.5 (11, 70)	39.5 (10, 74)	13.0 (0, 99)	30.0 (8, 61)
$P^{\&}$	-	0.734	<0.001*	0.002*	0.023*	0.010*
P^{*}	0.734	-	0.001*	0.006*	0.047*	0.025*
△ESR5/0, mm/h	-4.5 (-13, 1)	-2.5 (-6, 1)	-12.5 (-18, -4)	-7.0 (-16, -2)	-2.0 (-12, 0)	-6.0 (-11, -2)
$P^{\&}$	-	0.123	0.027*	0.219	0.523	0.900
P^{*}	0.123	-	<0.001*	0.006*	0.416	0.157
△CRP5/0, mg/dL	-4.5 (-18, 4)	-2.5 (-15, 4)	-11.5 (-29, -3)	-19.0 (-30, -1)	-17.0 (-36, -9)	-17.0 (-29, -3)
$P^{\&}$	-	0.705	0.001*	0.019*	0.001*	0.049*
P^{*}	0.705	-	0.019*	0.006*	<0.001*	0.019*
△BUN5/0, mg/dL	-2.5 (-7, 2)	-2.0 (-6, 1)	-3.5 (-8, 1)	-2.0 (-4, -1)	-1.5 (-4.5, 2)	1.5 (-2, 4)
$P^{\&}$	-	0.600	0.399	0.272	0.151	0.001*
P^{*}	0.600	-	0.156	0.600	0.384	0.005*
△Cr5/0, mg/dL	-0.05 (-0.2, 0.1)	-0.10 (-0.3, 0.1)	-0.10 (-0.2, 0.1)	-0.05 (-0.1, 0.1)	0.00 (-0.2, 0)	-0.05 (-0.2, 0.1)
$P^{\&}$	-	0.653	0.726	0.104	0.003	0.138
P^{*}	0.653	-	0.773	0.118	0.004*	0.155

Data were expressed as median (Q1, Q3); $P^{\&}$: compared with S group; P^{*}: compared with S+P group; S: hydroxychloroquine 200 mg twice per day, P: Placebo + hydroxychloroquine 200 mg twice per day, Arm 1: Single dose ivermectin (200 μg/kg), Arm 2: Three dose ivermectin (200 μg/kg), Arm 3: Single dose ivermectin (400 μg/kg), Arm 4: Three dose ivermectin (400, 200, 200 μg/kg).

Table 3. Clinical outcomes of the study patients.

Parameters	S	S+P	Arm 1	Arm 2	Arm 3	Arm 4	*P*-value	*H* or χ^2
Tachypnea Off (day)	2 (2, 3)	3 (2, 4)	2 (1, 3)	3 (2, 4)	3 (3, 5)	3 (3, 5)	0.584	27.884
$P^{\&}$	-	0. 689	0.162	0.143	0.023*	0.001*		
P^{*}	0. 689	-	0.001*	0.268	0.462	0.206		
Fever Off (day)	0 (0, 1)	0 (0, 1)	0 (0, 1)	0 (0, 1)	1 (1, 1)	1 (0, 2)	0.102	30.277
$P^{\&}$	-	0.753	0.498	0.597	<0.001*	0.128		
P^{*}	0.753	-	0.437	0.530	<0.001*	0.155		
Duration of low O_2 Sat	3 (2, 5)	4 (2, 6)	2 (1, 2)	3 (2, 5)	2 (1, 4)	5 (3, 6)	0.025*	41.933
$P^{\&}$	-	0.412	<0.001*	0.443	0.014*	0.074		
P^{*}	0.412	-	<0.001*	0.601	0.002*	0.233		
Duration on hospital stay (day)	7 (7, 9)	8 (6, 11)	6 (5, 7)	8 (6, 9)	5 (4, 7)	7 (6, 10)	0.006*	33.891
$P^{\&}$	-	0.658	0.012*	0.902	<0.001*	0.839		
P^{*}	0.658	-	0.005*	0.849	<0.001*	0.907		
Outcome								
Alive	25 (83.3)	24 (80.0)	30 (100.0)	27 (90.0)	30 (100.0)	29 (96.7)	0.001*b	18.64
Death	5 (16.7)	6 (20.0)	0 (0.0)	3 (10.0)	0 (0.0)	1 (3.3)		

S: hydroxychloroquine 200 mg twice per day, P: Placebo + hydroxychloroquine 200 mg twice per day, $P^{\&}$: compared with S group; P^{*}: compared with S+P group; Arm 1: Single dose ivermectin (200 μg/kg), Arm 2: Three dose ivermectin (200 μg/kg), Arm 3: Single dose ivermectin (400 μg/kg), Arm 4: Three dose ivermectin (400, 200, 200 μg/kg), Kruskal-Wallis *H* test was used to compare six groups, *Chi*-square was used for outcome.

among the six groups (*H*=33.891 *P*=0.006 and *H*=41.934, *P*=0.025 respectively). Regarding mortality, we observed a significant difference among the six groups. To further emphasize mortality reduction, we also calculated parameters such as risk in ivermectin group, risk in control group, overall risk, risk ratio, risk difference, prevented fraction in control group and prevented fraction in ivermectin group. First, the estimates from a single 2×2 table ("count" data) are presented followed by estimates adjusted or summarized across stratified data. Table 4 lists the risk of mortality for comparison between ivermectin treated and untreated groups with 95% *CI*. Our results, the estimation confirmed 15% reduction of mortality with risk ratio of 0.18.

Table 4. Risk of mortality in the study groups.

Type	Point estimates	95% *CI*
Risk in ivermectin groups	3.3%	(1.0%, 8.5%)
Risk in control groups	18.3%	(10.4%, 30.1%)
Overall risk	8.3%	(5.0%, 13.4%)
Risk ratio	0.18	(0.06, 0.55)*
Risk difference	-15.0%	(-25.3%, -4.7%)*
Prevented fraction in the control group	54.0%	(23.0%, 50.0%)
Prevented fraction in the ivermectin groups	81.8%	(45.3%, 94.0%)

Type: Taylor series, *95% confidence limits testing exclusion of 0 or 1, as indicated, *P*-values< 0.05 and confidence limits excluding null values (0, 1) are highlighted. The calculations in this table are based on group mergers. First, the estimates from a single 2 × 2 table ("count" data) were presented, then we calculated the parameters of Table 4 using statistical formulas. This table report risk of mortality in ivermectin treated and control groups. The estimation confirmed 15% reduction of mortality with risk ratio of 0.18.

4. Discussion

Totally, 180 patients infected with COVID-19 virus were allocated to six arms patients in the ivermectin group received single or interval doses of ivermectin. The positive effect of ivermectin therapy was observed in clinical consequences and patient recovery. The improvement of para-clinical outcomes of the patients was observed in ivermectin treated groups in comparison with standard and placebo arms. The mortality rate was comparable to the standard arms (16.7%) and placebo (20%). Since the beginning of the outbreak, various antiviral drugs such as Acyclovir, Ganciclovir, Oseltamivir, and Ribavirin have been tested. Unfortunately, these drugs have not been effective in curing or alleviating the symptoms of COVID-19[21]. Ivermectin, another FDA-approved drug, was reported by Caly *et al.* to inhibit the *in–vitro* replication of novel coronavirus. The results of Caly's study showed that a single treatment of ivermectin was able to induce a 5 000 folds reduction in the viral RNA 48 h in cell culture[11]. As suggested by previous studies, the initial disease intensity assessment cannot rely only on clinical examinations[22]. Our clinical trial was designed with features such as double-blind, placebo-controlled, multicenter, open-label, and randomized controlled trial. In this study, single and multiple oral doses (200 μg/kg and 400 μg/kg) of ivermectin were added to the standard regimen of patients (treatment protocols of Iranian Health Ministry for COVID-19). As illustrated in Table 2 and 3, the cure effect of ivermectin at different doses was confirmed by both laboratory and para-clinical parameters. The results of ivermectin treated subgroups comparing with the control group showed a difference between each subgroup and standard, placebo groups. The combined effects of different-doses of ivermectin on patients' medication regimens shortened the duration of hospitalization and low O_2 saturation. Promotion of other clinical parameters such as ALC, CRP, PLT and ESR showed that the ivermectin with a wide margin of safety had a higher therapeutic index and efficacy against COVID-19. One of the reported limitations of ivermectin in clinical utility is its potential to cause toxicity and studies have shown that this defect can be eliminated by changing the formulation and pharmacokinetic properties. Therefore, a systematic design based on concentration of ivermectin is essential[23]. Smitt *et al.* showed in a study based on the pharmacokinetic simulations that ivermectin may have limited therapeutic utility on controlling COVID-19. The reason is that the concentration of inhibitor required to act on the COVID-19 virus is much higher than the maximum plasma concentration by managing the approved dose; thus, they proposed ivermectin inhalation therapy[12,24,25]. However, they did not consider the host immune response in human and Clay *et al.* in their *in–vitro* study did not clarify if lower doses were effective or not. The antiviral properties of ivermectin have been demonstrated by targeting critical cellular process of mammalian cells. Therefore, in line with our study, it is speculated that ivermectin administration, even at low doses, can reduce the loading of the virus in the early stages[26]. In addition to above explanations, sustainability of ivermectin in different tissues of animals and human has been reported by various studies[27–29]. As mentioned in the introduction, it seems that the mechanisms of antiviral activity of ivermectin against viruses is mediated *via* targeting α / β -mediated nuclear transport of HIV-1 integrase and NS5 polymerase; NS3 helicase; nuclear import of UL42; and nuclear localization signal-mediated nuclear import of Cap[30–33]. On the other hand, a number of studies have emphasized the prophylaxis of ivermectin. For example, Cheng *et al.*, argued that it is useful as a preventative measure. Their study was performed to evaluate prophylaxis, especially after exposure to SARS-CoV-2 at a dose of 0.2 mg/kg body weight on the first day with a second additional dose of ivermectin on days 2 or 3 for older men[34]. In another study, ivermectin was given to high risk healthcare workers contact with COVID-19 patients. A comparison between the control and intervention group in the mentioned study showed that the symptoms of the disease decreased in the intervention group compared to the control group[35]. A randomized controlled study conducted by Boulware *et al.* reported no significant difference between the hydroxychloroquine arm and the placebo arm. There were also more side effects reported with hydroxychloroquine in their study[36]. Based on these findings, combined designs of ivermectin with other antiviral drugs appeared to have desirable results. In another study, the combination of ivermectin with hydroxychloroquine was investigated[37]. Using the mentioned doses of ivermectin given through this study, the follow-ups showed that there were no side effects such as nausea or skin rash during the trial and in overall our experience showed a good interaction between drug and patient. In a three arms phase 3 clinical trial, ivermectin was administered in two doses of 200 and 400 μg/kg in patients with dengue fever. The main purpose of this trial was to evaluate safety of ivermectin in children and adult patients and the results showed that one daily dose of ivermectin treatment for three days was safe[38]. In a retrospective study, the effects of standard dose of ivermectin (200 μg/kg) was assessed in sever COVID-19 patients in two trial arms including receiving and not receiving of the drug[39]. They reported no differences were found between these two arms and suggested the pursuing of the evaluations in higher doses of ivermectin through randomized clinical trials. There have been several reports about drug treatments that have significantly affected viral load in COVID-19 patients. The

novel coronavirus has several evolutionary phases, so that in the first clinical phase it causes the lower respiratory tract infection (LRTI) and upper respiratory tract infection (URTI) symptoms are associated with a high viral load that causes early lung lesions on LDCT[40,41]. Here, the focus of this study was to evaluate different dosing strategies of using ivermectin in COVID 19 disease to find its proper dose that may reduce the viral load and affect both LRTI and URTI symptoms. Moreover, because of the direct impact of ivermectin on the virus and its sustainability in different organs especially the pulmonary tissue, further investigations should be implemented to clarify the possible prophylactic effect of this drug against the first entrance of virus or after observation LRTI symptoms. In line with Rajter *et al*[42], which reported a 40% drop in mortality of critical COVID-19 patients after oral administration of ivermectin (200 μg/kg), as listed in Table 3 and 4, the lowest mortality rate was observed in the ivermectin treated arms in comparison with 16.7% and 20% rates in the standard and placebo plus standard arms respectively. The risk base estimation with 95% *CI* in Table 4 confirmed that the average mortality obtained in all of ivermectin treated arms was 3.3%, while it was about 18.3% in standard care and placebo arms. The significant improvement of clinical consequences such as the time of hospitalization (5 days), and duration of low O_2 saturation (2 days) were observed at the arms 1 and 3 with single dose of 200 and 400 μg/kg ivermectin, respectively. In comparsion with the other COVID-19 treatments such as Nitazoxanide[43], tocilizumab[44], stem cell therapy[45] and plasma therapy[46], which are costly and not affordable for the poor countries, this study propose ivermectin as a medicare with a wide safety margin, low cost and good efficacy for COVID-19 patients, thus, it is recommended for mild, moderate and even sever phases of disease.

The sample size was not large and the study was limited to the selected hospitals. Studies in areas with a maximum prevalence of COVID-19 and on patients with more diverse conditions such as body mass index over 40, different underlying diseases, or younger patients are needed. Another limitation was that some participants' disease was confirmed by a chest Image. Without these limitations, a stronger trial study could be carried out. Ongoing studies with larger sample sizes, confirmation of the disease of all participants through PCR , using strategies to enhance the antiviral potency of ivermectin and its combination with other antivirals or higher-dose regimens, and focus on severe COVID-19 cases are recommended.

Conflict of interest statement

We declare that we have no conflict of interest.

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Authors' contributions

GN, NMS, NP, AA designed the study. BB,VM,YYMJ, KA, FA, KM and JR were responsible for overall data collection and clinical studies. ZL, JA, AA conducted data analysis and interpretation. ZL drafted the first manuscript and has designed Figure1. GN and AA revised the manuscript critically. NY and ZL were in charge of drug formulation. All authors read and approved the final manuscript. NMS,GN,CHF and NP supervised the project.

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