
EVALUATION OF THE EFFECTIVENESS AND SAFETY OF ADDING IVERMECTIN TO TREATMENT IN SEVERE COVID-19 PATIENTS

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STUDY PROTOCOL

Introduction and Aim

Since the first case was reported in Wuhan, China in December 2019, the SARS CoV-2-induced COVID19 epidemic, which has surrounded the whole world with great speed, still continues its effect as a pandemic. According to the first reports, SARS CoV-2, which was reported to originate from the live animal market in Wuhan and has been associated with two bat corona viruses so far, has also gained the ability to be transmitted from person to person and spread all over the world. SARS CoV-2 is an enveloped, single-stranded betacoronavirus. The virus is mainly found in the respiratory secretions of patients and it is transmitted to susceptible people by droplets as a result of the spillage of these secretions into the environment. However, to date, it has been shown that the virus is also found in the gastrointestinal canal, feces and urine, and it has been reported that it is necessary to investigate whether it is transmitted by these means (1,2).

In a meta-analysis that evaluated nine studies and included a total of 50,404 patients, the main symptoms seen in COVID19 were reported as high fever, cough, myalgia and fatigue. In the same publication, a severe picture with acute respiratory failure is defined in 14.8% of the patients, and the average mortality is reported as 4.8% (3). In the diagnosis of the disease, lung tomography findings, which are compatible with the clinic, are particularly important (4).

The gold standard for microbiological diagnosis of the disease is to search for virus RNA in respiratory tract samples by real-time PCR. However, these tests are likely to give false negative results. In this case, radiological findings and some hematological parameters are used for diagnosis. It has been reported that most of the patients have lymphopenia, high CRP and d-dimer levels, and procalcitonin levels are close to normal. For faster diagnosis, rapid diagnostic tests have been developed to detect IgM / IgG type antibodies in blood samples of patients, and there are studies conducted to determine the sensitivity and specificity of these tests (1,3).

There is no drug with proven efficacy in the treatment of the disease. Ribavirin, hydroxychloroquine, lopinavir-ritonavir, remdesivir, favipiravir, azithromycin are among the drugs being tried. In patients with severe prognosis and with septic shock, cytokine antagonists such as corticosteroids, tocilizumab, and plasma treatments obtained in recovered patients are tried (5,6).

Ivermectin, which is an antiparasitic drug effective against endo and ecto parasites in the structure of 22,23 dihydroavermectin B1, which is produced semi-synthetically from the avermectin family, has been approved by the FDA for oral use in humans, it is a highly effective drug for ascariasis, cutaneous larval migrans, strongiloidosis, onchocerciasis, and scabies (7).

In addition to its antiparasitic activity, ivermectin has been shown in in vitro studies in recent years that it has antiviral efficacy against many viruses such as human immunodeficiency virus (HIV-1), dengue virus and western Nile virus. In a phase 3 study between 2014 and 2017 in people with Dengue virus infection in Thailand, it was reported that orally once a day was used and it was safe, and it did not provide clinical improvement, although a significant decrease was observed in the serum viral NS1 protein level (8, 9).

The most common side effects during ivermectin treatment; fever, headache, dizziness, itching and rash. Rarely, neurological side effects such as encephalopathy, confusion and coma have been reported during the use of ivermectin in the treatment of onchocerciasis. These side effects have been shown in animal and human studies to be related to the mdr-1 gene mutation. It has been shown that in persons carrying the SNP (single nucleotide polymorphisms) mutation in the mdr-1 / abcb1 gene encoding P-glycoprotein, this gene expression decreases and ivermectin passes to the central nervous system and accumulates and leads to encephalopathy.

In addition, it has been reported that haplotypes and mutations in the CYP3A4 Gene that give and lose function cause toxic effects or drug dosing by changing the metabolic rate of ivermectin.

In a recently published in vitro study from Australia, the efficacy of ivermectin used as an antiparasitic agent on SARS-CoV-2 was evaluated and Vero/hSLAM cells were infected with this virus in vitro and exposed to ivermectin, after 48 hours there was a 99.8% reduction in viral load. Based on these studies, it can be thought that ivermectin, which has also been shown to have antiviral efficacy in different studies, may also be effective in COVID19 patients (8).

In this study, it is aimed to evaluate the effect of ivermectin addition to the "Hydroxychloroquine ± Favipiravir ± Azithromycin" combination therapy, which is the reference treatment recommended in the "Covid19 Guideline" prepared by the Ministry of Health, on clinical response in patients with severe COVID19 pneumonia and to investigate the safety of ivermectin use in these patients (10).

Material and Method

The study will be conducted as a prospective, randomized, controlled, single-blind Phase 3 study. Patients with severe COVID19 disease who were hospitalized in Afyonkarahisar Health Sciences University, Health Sciences University Istanbul Sultan Abdülhamid Han Training and Research Hospital, Ankara City Hospital and Health Sciences University Ankara Gülhane Training and Research Hospital COVID19 service and intensive care units will be included in the study.

There will be two patient groups in the study, the study arm and the control arm. A total of 60 patients, including 30 in both arms, are planned to be included in the study.

Patient Selection and Inclusion Criteria

Patients who were hospitalized with a pre-diagnosis of *severe COVID-19 pneumonia and thereafter diagnosis of COVID-19 was also confirmed microbiologically with PCR positivity in respiratory tract samples were included into the study (13). They were randomized to the study and control group, respectively.

** Patients with at least one of the criteria below were accepted as patients with severe COVID-19 pneumonia;*

- a. Presence of tachypnea ≥ 30 /minute, SpO₂ level $< 90\%$ in room air, PaO₂/FiO₂ < 300 in oxygen receiving patient
- b. Presence of specific radiological finding for Covid-19 in lung tomography (bilateral lobular, peripherally located, diffuse patchy ground glass opacities)
- c. Mechanical ventilation requirement
- d. Acute organ dysfunction findings; patients with SOFA (sepsis-related organ failure assessment) score > 2

THE SEQUENTIAL ORGAN FAILURE ASSESSMENT (SOFA) SCORE

SYSTEM	0	1	2	3	4
Respiration PaO ₂ /FIO ₂ mm Hg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation Platelets ×10 ³ /uL	≥150	<150	<100	<50	<20
Liver Bilirubin mg/dL (umol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP ≥70mmHg	MAP <70mmHg	Dopamine <5 or Dobutamine (any dose)	Dopamine 5.1 - 15 or Epinephrine ≤ 0.1 or Norepinephrine ≤ 0.1	Dopamine >15 or Epinephrine >0.1 or Norepi- nephrine >0.1
CNS GCS Score	15	13-14	10-12	6-9	<6
Renal Creatinine, mg/dl (umol/L) Urine Output, ml/d	<1.2 (110)	1.2 -1.9 (110-170)	2.0 - 3.4 (171- 299)	3.5 - 4.9 (300 -440) <500	> 5.0 (440) <200

Catecholamine Doses = ug/kg/min for at least 1hr

Mutations in the *mdr1 / abcb1* gene and the *CYP3A4* gene will be investigated in patients who meet any of the above criteria and are > 18 years of age and enrolled in the study group by randomization. Patients with mutations will not be included in the study.

Exclusion Criteria

Patients with the following characteristics were excluded from the study.

1. Pediatric patients; <18 years of old
2. Patients with chronic liver or kidney disease
3. Pregnant women
4. Patients with known ivermectin allergy

Genetic study

In the patients included in the study group, the mutation in 29 pairs of primers and the haplotypes and mutations of the CYP3A4 gene will be investigated by performing sequence analysis in the *mdr1* / *abcb1* gene with the Sanger method.

Design of Primers

"PRIMER © – Primer Designer v.2.0 (Scientific & Educational Software)" software will be used in primer design for mutation research. Primers will be synthesized by Metabion International AG. Sequence analysis plan and primer design will be made, including the coding regions of these genes, exon-intron junction regions.

Sanger Sequence Analysis

DNA fragments for all patients will be sequenced with the ABI 3130 sequence analyzer after amplification using polymerase chain reaction (PCR) followed by bi-directional fluorescent markers using BigDye chemistry (Life Technologies, Carlsbad, CA, USA). The sequences will be read with the Seqscape program. Variants obtained here will be analyzed according to ACMG criteria and variants will be classified. In the analysis of variants, data will also be evaluated using relevant databases to identify known haplotypes.

Treatment and patient follow-up

The following reference treatment recommended by the Ministry of Health will be applied to all patients in the control and study groups;

1. Hydroxychloroquine (2x400mg loading dose followed by 2x200mg, po, 5 days)
2. Favipiravir (2x1600mg loading followed by 2x600mg, po, maintenance dose for a total of 5 days)
3. Azithromycin (500mg first day followed by 250mg / day 4 days, po, 5 days total)

In addition to the reference therapy, ivermectin at a dose of 200 mcg / kg / day (9 mg between 36-50 kg, 12 mg between 51-65 kg, 15 mg between 66-79 kg, > 80 kg 200mcg / kg) It will be applied for five days in the form of a solution prepared for enteral use (Ivermectin 5mg/5ml solution was manufactured by Neutec Pharmaceutical-Turkey, under GMP conditions).

Patients in the control arm will be given reference treatment with the other 3 drugs without giving ivermectin.

The drug whose effectiveness will be evaluated in the study is ivermectin. Ivermectin is an FDA-approved antiparasitic drug. Approved adult dose in parasitic infections is reported as 200mcg / kg / day (11). Although ivermectin has been shown to be effective in SARS CoV-2 in vitro, there is no randomized prospective controlled study showing that it is effective in COVID19 patients. In a very recent study that is still in press, it has been reported that it significantly reduces mortality in COVID19 patients, and in this study, no information is given about the duration and dosage of use (12).

In a study conducted in Thailand on the use of ivermectin in dengue fever infection, it was reported that it was used at doses of 200mcg / kg / day and 400mcg / kg / day (13).

Ivermectin antiparasitically as a single daily or multi-dose intermittent sequential; In dengue fever infection, considering the use of 200 or 400 mcg / kg / day as an antiviral for 3 days, it is seen that the total dose given in antiviral treatment reaches up to 1200 mcg / kg / day (14,15,16). Based on all these studies, we planned to use ivermectin at a dose of 200 mcg / kg / day, which is approved by the FDA, in our study. Definitive recommendations for treating COVID19 disease Although not, the duration of treatment with antiviral drugs is recommended as 5 days in the guide prepared by the Scientific Committee of the Ministry of Health. Considering that the average duration of symptoms in patients is 5-7, the duration of ivermectin treatment was planned as 5 days in our study, and it was planned to reach a total dose of 1000 mcg / kg / day, close to the total dose used in viral infections in the literature. The treatment can be terminated in less than 5 days if the patients reach the treatment early or if there are side or toxic effects.

Study Design

In all patients included in the study; demographic data, comorbid conditions, admission symptoms, complete blood count and biochemical blood examinations at the time of admission, initial SARSCoV-2 PCR results and thoracic tomography findings at presentation will be recorded.

During the study; respiratory findings and laboratory parameters of the patients will be recorded on the 1st, 3rd and 5th days of the treatment and on the 1st, 3rd and 5th days after the treatment, during the follow-up period. Side effects observed during the treatment in all patients also will be recorded.

Primary and secondary endpoints for efficacy and safety assessment in the study will be determined as follows:

Primary endpoint: Clinical responses and drug side effects obtained in patients on the 5th day, at the end of the ivermectin treatment will be evaluated. Extubation in mechanically ventilated patients, respiratory rate <26 , SpO₂ level in room air $>90\%$, PaO₂ / FiO₂ >300 in patients receiving oxygen, presence of at least two of the 2-point reduction criteria in SOFA score will be evaluated as "*clinical response*".

Secondary endpoint: Clinical responses and drug side effects obtained in patients on the 5th day after the end of ivermectin treatment will be evaluated. For clinical response, the presence of at least two of the following criteria is sought: Respiration rate between 22-24, SpO₂ level in room air $>95\%$, absence of oxygen requirement, observation of radiological improvement in control lung tomography, no need for intensive care.

In order to evaluate the treatment response in patients; blood lymphocyte count, CRP, ferritin and d-dimer values, changes in polymorphonuclear leukocyte/lymphocyte (PNL/L) ratio, changes in SpO₂ and PaO₂/FiO₂ ratio will be determined and compared between both groups. Mortality rates at the end of the follow-up period will be also evaluated in both groups.

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