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Original Article

# Effect of minocycline in COVID-19 patients: A randomized clinical trial

**Danial Esmaeilzadeh[1], Mohammad Shariati Rad[1], Mehrdad Esmaeilzadeh[2], Bibi Marjan Razavi[3,4], Amirhooshang Mohamadpoor[5,6*], Hossein Hosseinzadeh[4,6*]**

[1]School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Infectious Disease, Hasheminejad Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Department of Clinical Pharmacy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[6]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

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**Abstract**

**Introduction:** Minocycline a semi-synthetic tetracycline is a potential therapeutic option for COVID-19 because of its anti-inflammatory and immunomodulatory effects. Moreover, its antioxidant, antiviral, and antiapoptotic effects have been proven. In this study, the efficacy of minocycline in the therapy of COVID-19 patients has been evaluated.

**Methods:** A randomized double-blind placebo-controlled clinical trial was performed in Mashhad, Iran. 40 outpatients were randomized to either the treatment with the minocycline group or the placebo group, in a 1:1 ratio with 20 patients in each group. The Iranian National COVID-19 Therapy Regimen at the time was used in both groups and patients in the treatment group also received oral minocycline 100 mg twice day for 14 days. Patients in both groups were followed on days 3, 7, and 14 after initiating therapy for clinical symptom improvement, improvement of lymphocytes, leukocytes, C-reactive protein (CRP) and SpO2.

**Results:** A total of 40 patients with similar demographic and disease characteristics were enrolled. Results showed that the time interval until clinical symptoms improvement was significantly reduced in the minocycline group (6.85 ± 0.79, day) compared to the placebo (10.95 ± 1.18, day) group ($P = 0.006$). Moreover, the time interval until leukocytes reaching normal limits was significantly reduced in the minocycline group (3.95 ± 0.59, day) compared to the placebo (6.72 ± 1.25, day) group ($P = 0.046$).

**Conclusion:** In this randomized double-blind placebo-controlled study, minocycline (100 mg, BID for 14 days) reduced the duration of clinical symptoms improvement as well as the duration of white blood cell (WBC) normalizing in outpatients of COVID-19 disease.

## Introduction

A series of acute atypical respiratory diseases was found in Wuhan, China in December 2019 which is now termed SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) because of high homology to SARS-CoV (~80%) that triggered acute respiratory distress syndrome (ARDS). The disease caused by this virus is now called COVID-19. This virus primarily affects the respiratory system and other organs are also involved.[1,2]

The clinical varieties of the disease include mild, moderate, or severe illnesses. Many patients are asymptomatic carriers who have the potential to infect others or have a mild illness that cannot be differentiated from a simple upper respiratory tract infection. Moderate and severe cases need hospitalization and intensive therapy including invasive and non-invasive ventilation, together with antivirals, antibiotics, antipyretics, and steroids. Immunomodulatory drugs and plasma exchange therapy are also required for complicated cases.[3] According to documents, the time between the onset of symptoms and the development of ARDS is about 9 days. Respiratory symptoms of COVID-19 are very heterogeneous, from minimal symptoms to significant hypoxia with ARDS. The most common symptoms of the disease are fever, malaise, body pain, dry cough, dyspnea, and gastrointestinal symptoms (in some patients) such as diarrhea, vomiting, and abdominal pain. Similar to ARDS, the complications of the disease are due to cytokine storms.[3-5] This disease is formed of two phases

***Corresponding Author:** Amirhooshang Mohamadpoor, Email: mohamadpoorAH@mums.ac.ir; Hossein Hosseinzadeh, Email: hosseinzadehh@mums.ac.ir

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including asymptomatic and symptomatic phases. The asymptomatic phase lasts about several days and has a limited immune response with low viral loads, however, the affected individuals are contagion.[3]

In the symptomatic phase, about one-fifth of patients have the chance of severe disease progression as ARDS due to the cytokine storm, in which infected pneumocytes release cytokines and inflammatory markers such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-12 (IL-12), tumor necrosis factor α (TNF-α), interferon-gamma (IFN-γ), C–X–C motif chemokine 10 (CXCL-10), and monocyte chemoattractant protein-1 (MCP-1). These cytokines attract neutrophils, the cluster of differentiation 4 (CD-4) T, and the cluster of differentiation 8 (CD-8) T cells that are necessary for viral body defense but they cause inflammation and pulmonary damage in pathological levels.[6]

No targeted therapy is available for COVID-19 suffering people and the treatment is based on supportive therapy. Some drugs such as remdesivir, lopinavir-ritonavir, hydroxychloroquine, and azithromycin have been used,[7-10] but none of them have been proven to be a definite therapy yet.[2,11]

Minocycline is a semi-synthetic antibiotic that belongs to the second generation of tetracycline-class which is prescribed in infectious and non-infectious diseases.[12] This agent crosses the brain-blood barrier, therefore, it has the most neuroprotective effects among tetracyclines. The bacteriostatic effect of minocycline is due to the binding to the 30s ribosomal subunit, therefore, and blocking protein synthesis.[12]

Bacteria susceptible to minocycline include *Mycoplasma pneumoniae*, *Staphylococcus aureus* (including methicillin-resistant *Staphylococcus aureus*), *Borrelia recurrentis*, *Mycobacterium marinum*, *Acinetobacter baumannii*, *Vibrio vulnificus*, and susceptible strains of vancomycin-resistant enterococcus. Minocycline is used for acne, nocardiosis, brucellosis, ehrlichiosis, amebiasis, actinomycosis, anaplasmosis, leptospirosis, melioidosis, tularemia, traveler's diarrhea, Lyme disease (early stage), legionnaire's disease, Whipple disease, rickettsial infections, chlamydial infections, syphilis and pelvic inflammatory diseases.[13]

Beside antibiotic effects, minocycline possesses antioxidant, anticancer, anti-inflammatory, immunomodulatory, antiviral, and antiapoptotic properties.[14,15]

Minocycline is used in non-infectious diseases such as aortic aneurysms, cancer metastasis, periodontitis, bullous dermatoses, neutrophilic diseases, pyoderma gangrenosum, sarcoidosis, rosacea, as well as autoimmune disorders such as rheumatoid arthritis and scleroderma.[16,17]

According to documents, it has antiviral effects in both in vitro and in vivo studies. Its antiviral effects against human immunodeficiency virus (HIV) have been shown. Minocycline leads to the weakening of HIV infection by reducing C-C chemokine receptor type 5 (CCR5) expression and interfering with the HIV integrase enzyme that is necessary for entering and replication of HIV in cells.[18] Additionally, in some in vitro studies efficiency of minocycline against influenza, rabies, human T-cell leukemia virus type 1 (HTLV1), reovirus, Japanese encephalitis, and West Nile has been proved.[19] Moreover, it has protective effects and reduces cell damage against the respiratory syncytial virus (RSV).[20]

By inactivation of microglia, inhibition of inflammatory mediators such as IL-6, IL-2, and TNF-α, phagocytes inactivation and inhibiting the expression of IFN-γ on CD8-T cells, minocycline suppresses the immune system which results in anti-inflammatory outcomes.[16,21-23]

Due to the lack of effective drug therapy for COVID-19 until now, searching for new therapies is an effective step for the treatment of this disease. According to the potential anti-inflammatory effects of minocycline, as well as its antioxidant, immunomodulatory, anti-apoptotic, and antiviral effects, it is expected that this drug may be useful for COVID-19 therapy or reducing the duration of treatment. So, in this clinical trial, the effect of minocycline in the treatment of COVID-19 was evaluated.

## Materials and Methods

### *Study type*

This randomized, double-blind, placebo-controlled, clinical trial was conducted to investigate the effect of minocycline in COVID-19 patients. The study was performed between April 2020, and February 2021, in the Hasheminejad hospital in Mashhad, Khorasan Razavi, Iran.

### *Patient population, inclusion, and exclusion criteria*

This study was conducted on 40 adult outpatients (aged ≥ 18 years) who were confirmed by infectious disease specialists based on clinical and laboratory findings. The subjects, based on inclusion and exclusion criteria, were randomized (20 persons per group) (Figure 1). Inclusion criteria were the following: Patients with COVID-19 symptoms who were selected for home quarantine and outpatient medication with the age range of 18 to 65 years in both genders and signed informed consent. Exclusion criteria were the following: patients connected to a catheter, patients under chemotherapy, patients taking cytotoxic drugs or corticosteroids, pregnant and lactating patients, patients with severe renal insufficiency, liver failure, diabetes, autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, etc). An esophageal ulcer is common with taking minocycline, so, patients should take at least one glass of water in a standing position and before going to bed. Also, because of its interaction with divalent and trivalent cations such as iron, it is advised to the patient to gap 3 to 4 hours between taking minocycline and these complements.

### *Drug dosing and administration*

Patients voluntarily signed the form developed for this

trial. The informed consent forms were approved by the research ethics committee of Mashhad University of Medical Sciences (MUMS). Participants were randomized according to a random number table, to remove potential effects of confounding factors. Patients are divided randomly into two groups including minocycline and placebo receivers. Both of them received routine COVID-19 drug therapy based on the Iranian national COVID-19 treatment protocol and best practice guidelines at that time. Moreover, the drug group additionally received minocycline 100 mg twice a day and the placebo group received a placebo (starch) twice a day for two weeks.

### *Outcome measures*

Demographic data, medical history, and accurate physical examination were recorded at the time of admission. Patients in both groups were followed on days 3, 7, and 14 after initiating therapy for clinical symptoms improvement (such as fever, dyspnea, myalgia, cough, gastrointestinal symptoms, etc) as well as improvement of lymphocytes, leukocytes, C-reactive protein (CRP), and $SpO_2$. Clinical symptoms were ascertained by a clinical pharmacist blinded to the treatment allocation. Clinical symptoms were recorded as mild, moderate, and severe according to each criterion. A structured questionnaire for admission baseline and days 3, 7, and at the end of the treatment period was used.

### *Blinding and randomization*

Placebos were matched in details such as color, size, and packaging with the minocycline and coded by a medical student. All people including patients, physicians, researchers and nurses were unaware of the code written on the capsule boxes. Randomization was performed using computer-generated random numbers. Researchers and physicians were not informed about randomization and allocation until finishing the analyses. A trained nurse was responsible for the randomization of patients and allocating them into mentioned groups in the hospital.

### *Statistical analysis*

Mean ± SEM and grading (mild, moderate, and severe) were used for reporting quantitative and qualitative variables, respectively.

Normally and non-normally distributed quantitative data were identified by the Shapiro-Wilk test and then compared between groups by independent samples *t* test and Mann-Whitney test.

The qualitative variables were compared between two groups by the chi-square test. Repeated measures ANOVA was used to compare laboratory findings such as CRP, leukocytes, lymphocytes and $SpO_2$ between two groups on different days of 0, 3, 7, and 14. Statistical analyses were conducted using SPSS software (version 21, SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was considered significant.

## Results

### *Patient baseline characteristics*

A total of 120 patients with proven COVID-19 disease were assessed at Hasheminejad hospital. Among them, 73 did not meet the criteria for entering the study, 4 did not tend to participate in the study and 3 of the patients were far from the hospital who were not able to complete the study. Eventually, 40 patients were assigned to this clinical trial. Patients were randomly divided into treatment and placebo groups by a 1:1 ratio with 20 in each group. The baseline variables of patients participating in the study were balanced between treatment and control groups to indicate a typical population of patients with COVID-19 disease. Demographic, laboratory findings, and disease severity between patients of these groups were shown to be similar by statistical analysis (Table 1). The average age was 46.4 ± 3.22 years among the treated group and 43.75 ± 2.43 years in the control group. In terms of gender, 18 were female and 22 were male and the percentages of males in the treatment and placebo group were 50% and 60%, respectively.

### *Effect of minocycline on clinical symptoms*

A non-significant reduction in fever was found in the minocycline group compared to the placebo group at days 3 ($P = 0.244$) (Table 2), 7 ($P = 0.064$) (Table 3), and 14 ($P = 0.223$) (Table 4) after initiating therapy. No significant differences were found in dyspnea, malaise, cough and myalgia between placebo and treatment groups at days 3 ($P = 0.413$, $P = 0.744$, $P = 0.827$ and $P = 0.177$) (Table 2), 7 ($P = 0.233$, $P = 0.958$, $P = 0.241$ and $P = 0.571$) (Table 3) and 14 ($P = 0.558$, $P = 0.392$, $P = 0.052$ and $P = 0.935$) (Table 4), respectively. Other clinical symptoms and their severity are shown in Tables 2-4 on days 3, 7, and 14.

### *Effect of minocycline on time interval until clinical symptoms improvement*

A significant reduction in the time interval for the improvement of clinical symptoms was seen in the minocycline receivers (6.85 ± 0.79, day) compared to the placebo (10.95 ± 1.18, day) group ($P = 0.006$) (Table 5).

### *Effect of minocycline on SpO2*

There was no significant difference in SpO2 analyzed by repeated-measures ANOVA on days 0, 3, 7, and 14 between the two groups (Figure 2C).

### *Effect of minocycline on time interval until SpO2 reaching the normal limits*

There was no significant difference between time intervals until CRP reaching the normal limits among treatment and placebo groups ($P = 0.112$) (Table 5).

### *Effect of minocycline on White blood cells (WBCs), lymphocytes, and CRP*

There were no significant differences in CRP (Figure 2A),

**Table 1.** Demographic and clinical characteristics in patients treated with minocycline and placebo group (day 0)

| | Placebo group (n = 20) | Treatment group (n = 20) | *P* value |
|---|---|---|---|
| **Demographic characteristics** | | | |
| Age (y) | 43.75 ± 2.43 | 46.40 ± 3.22 | 0.516[a] |
| Gender (male/female) | 12/8 | 10/10 | 0.751[b] |
| **Clinical characteristics** | | | |
| Fever (°C) | 36.71 ± 0.15 | 36.78 ± 0.13 | 0.747[a] |
| $SpO_2$ (%) | 95.44 ± 0.95 | 94.38 ± 1.08 | 0.466[a] |
| WBC ($10^3$/uL) | 5.21 ± 0.58 | 6.71 ± 0.53 | 0.078[a] |
| Lymphocytes/uL | 1337 ± 149 | 1986 ± 283 | 0.055[a] |
| Cough (No.) | Mild: 5 | 3 | 0.099[b] |
| | Moderate: 2 | 9 | |
| | Severe: 3 | 3 | |
| | Total: 10 | 15 | |
| Dyspnea (No.) | Mild: 2 | 4 | 0.413[b] |
| | Moderate: 1 | 3 | |
| | Severe: 6 | 7 | |
| | Total: 9 | 14 | |
| Malaise (No.) | Mild: 5 | 9 | 0.116[b] |
| | Moderate: 7 | 5 | |
| | Severe: 1 | 4 | |
| | Total: 13 | 18 | |
| Chest pain (No.) | Mild: 4 | 5 | 0.96[b] |
| | Moderate: 4 | 3 | |
| | Severe: 1 | 1 | |
| | Total: 9 | 9 | |
| Body pain (No.) | Mild: 1 | 5 | 0.174[b] |
| | Moderate: 2 | 4 | |
| | Severe: 7 | 3 | |
| | Total: 10 | 12 | |
| Headache (No.) | Mild: 4 | 3 | 0.83[b] |
| | Moderate: 4 | 3 | |
| | Severe: 4 | 4 | |
| | Total: 12 | 10 | |
| Diarrhea (No.) | Mild: 4 | 5 | 0.772[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 4 | 5 | |
| Nausea (No.) | Mild: 6 | 3 | 0.509[b] |
| | Moderate: 2 | 2 | |
| | Severe: 1 | 0 | |
| | Total: 9 | 5 | |
| Vomiting (No.) | Mild: 2 | 1 | 0.515[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 2 | 1 | |
| Abdominal Pain (No.) | Mild: 6 | 1 | 0.177[b] |
| | Moderate: 1 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 8 | 3 | |
| Loss of taste (No.) | Mild: 0 | 1 | 0.466 |
| | Moderate: 0 | 0 | |
| | Severe: 4 | 6 | |
| | Total: 4 | 7 | |
| Loss of smell (No.) | Mild: 1 | 0 | 0.565[b] |
| | Moderate: 0 | 0 | |
| | Severe: 6 | 6 | |
| | Total: 7 | 6 | |
| Rhinorrhea (No.) | Mild: 5 | 2 | 0.277[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 1 | |
| | Total: 5 | 3 | |
| Sore throat (No.) | Mild: 4 | 3 | 0.694[b] |
| | Moderate: 0 | 1 | |
| | Severe: 1 | 2 | |
| | Total: 5 | 6 | |
| Sweating (No.) | Mild: 6 | 3 | 0.462[b] |
| | Moderate: 4 | 4 | |
| | Severe: 5 | 5 | |
| | Total: 15 | 12 | |
| Anorexia (No.) | Mild: 4 | 2 | 0.548[b] |
| | Moderate: 6 | 6 | |
| | Severe: 4 | 3 | |
| | Total: 14 | 11 | |
| Chill (No.) | Mild: 6 | 8 | 0.725[b] |
| | Moderate: 3 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 10 | 10 | |

$SpO_2$: saturation of peripheral oxygen, No.: number of patients, CRP: C-reactive protein, WBCs: White blood cells.
[a] Independent samples t-test, [b] Chi-squared test.

lymphocytes (Figure 2B), and WBC (Figure 2D) analyzed by repeated-measures ANOVA on days 0, 3, 7, and 14 between the two groups.

Although patients in the treatment group showed improvement in the level of WBCs between days 0, 3, 7, and 14 compared to the placebo group, however, the improvement was not significant ($P = 0.055$) (Figure 2D).

### *Effect of minocycline on time interval until WBCs reaching the normal limits*

The time interval until leukocytes reaching the normal limits was significantly reduced in the minocycline group (3.95 ± 0.59, day) compared to the placebo (6.72 ± 1.25, day) group ($P = 0.046$) (Table 5).

### *Effect of minocycline on time interval until CRP reaching the normal limits*

There was no significant difference between time intervals

**Table 2.** Clinical characteristics in patients treated with minocycline and placebo group (day 3)

| | Placebo group (n = 20) | Treatment group (n = 20) | *P* value |
|---|---|---|---|
| Fever (℃) | 36.12 ± 0.32 | 35.39 ± 0.51 | 0.244[a] |
| SpO2 (%) | 95.59 ± 0.42 | 94.60 ± 0.72 | 0.252[a] |
| CRP (mg/l) | 11.71 ± 4.27 | 14.28 ± 5.19 | 0.705[a] |
| WBC ($10^3$/ul) | 6.45 ± 0.51 | 6.76 ± 0.39 | 0.642[a] |
| Lymphocytes/ul | 2384 ± 194 | 2148 ± 152 | 0.34[a] |
| Cough (No.) | Mild: 3 | 9 | 0.827[b] |
| | Moderate: 1 | 1 | |
| | Severe: 0 | 0 | |
| | Total: 4 | 10 | |
| Dyspnea (No.) | Mild: 2 | 6 | 0.413[b] |
| | Moderate: 5 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 8 | 8 | |
| Malaise (No.) | Mild: 6 | 4 | 0.744[b] |
| | Moderate: 2 | 4 | |
| | Severe: 2 | 3 | |
| | Total: 10 | 11 | |
| Chest pain (No.) | Mild: 4 | 5 | 0.555[b] |
| | Moderate: 1 | 0 | |
| | Severe: 0 | 1 | |
| | Total: 5 | 6 | |
| Body pain (No.) | Mild: 1 | 2 | 0.177[b] |
| | Moderate: 1 | 1 | |
| | Severe: 0 | 4 | |
| | Total: 2 | 7 | |
| Headache (No.) | Mild: 3 | 3 | 0.47[b] |
| | Moderate: 2 | 6 | |
| | Severe: 2 | 1 | |
| | Total: 7 | 10 | |
| Diarrhea (No.) | Mild: 5 | 6 | 0.996[b] |
| | Moderate: 1 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 7 | 8 | |
| Nausea (No.) | Mild: 6 | 3 | 0.264[b] |
| | Moderate: 1 | 3 | |
| | Severe: 0 | 2 | |
| | Total: 7 | 8 | |
| Vomiting (No.) | Mild: 1 | 2 | 0.486[b] |
| | Moderate: 0 | 1 | |
| | Severe: 0 | 1 | |
| | Total: 1 | 4 | |
| Abdominal Pain (No.) | Mild: 1 | 3 | 0.372[b] |
| | Moderate: 1 | 3 | |
| | Severe: 1 | 0 | |
| | Total: 3 | 6 | |
| Loss of taste (No.) | Mild: 3 | 6 | 0.764[b] |
| | Moderate: 1 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 5 | 8 | |
| Loss of smell (No.) | Mild: 2 | 5 | 0.401[b] |
| | Moderate: 0 | 0 | |
| | Severe: 3 | 4 | |
| | Total: 5 | 9 | |
| Rhinorrhea (No.) | Mild: 2 | 0 | 0.134[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 2 | 0 | |
| Sore throat (No.) | Mild: 0 | 1 | 0.514[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 0 | 1 | |
| Sweating (No.) | Mild: 5 | 7 | |
| | Moderate: 0 | 1 | |
| | Severe: 4 | 1 | |
| | Total: 9 | 9 | |
| Anorexia (No.) | Mild: 4 | 9 | 0.117[b] |
| | Moderate: 2 | 0 | |
| | Severe: 1 | 3 | |
| | Total: 7 | 12 | |
| Chill (No.) | Mild: 1 | 4 | 0.271[b] |
| | Moderate: 1 | 0 | |
| | Severe: 0 | 1 | |
| | Total: 2 | 5 | |

$SpO_2$: saturation of peripheral oxygen, No.: number of patients, CRP: C-reactive protein, WBCs: White blood cells.
[a] Independent samples t-test, [b] Chi-squared test.

until CRP reaching the normal limits among treatment and placebo groups ($P = 0.478$) (Table 5).

### *Adverse events*

No major adverse events were found except for some reports of nausea just after taking the capsules in both groups.

## Discussion

The data presented in this clinical trial showed that minocycline 100 mg, BID significantly reduced the duration of clinical symptoms improvement and duration of WBC normalizing compared to the placebo group.

The clinical presentation of COVID-19 involves a broad range, from asymptomatic infection to a severe fatal disease. The most common symptoms of COVID-19 include fever, cough, fatigue, and dyspnea which are consistent with the common symptoms of a viral infection and pneumonia.[24]

**Table 3.** Clinical characteristics in patients treated with minocycline and placebo group (day 7)

| | Placebo group (n = 20) | Treatment group (n = 20) | *P* value |
|---|---|---|---|
| Fever (°C) | 36.194 ± 0.26 | 35.153 ± 0.46 | 0.064[a] |
| $SpO_2$ (%) | 95.44 ± 0.39 | 95.53 ± 0.44 | 0.888[a] |
| CRP (mg/L) | 11.41 ± 3.68 | 6.35 ± 2.36 | 0.257[a] |
| WBC ($10^3$/ul) | 5.98 ± 0.39 | 6.22 ± 0.33 | 0.656[a] |
| Lymphocytes/ul | 2269 ± 177 | 2119 ± 109 | 0.47[a] |
| Cough (No.) | Mild: 5 | 9 | 0.241[b] |
| | Moderate: 0 | 1 | |
| | Severe: 0 | 0 | |
| | Total: 5 | 10 | |
| Dyspnea (No.) | Mild: 3 | 8 | 0.233[b] |
| | Moderate: 1 | 1 | |
| | Severe: 0 | 0 | |
| | Total: 4 | 9 | |
| Malaise (No.) | Mild: 6 | 7 | 0.958[b] |
| | Moderate: 2 | 3 | |
| | Severe: 1 | 1 | |
| | Total: 9 | 11 | |
| Chest pain (No.) | Mild: 3 | 2 | 0.481[b] |
| | Moderate: 1 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 4 | 2 | |
| Body pain (No.) | Mild: 1 | 1 | 0.571[b] |
| | Moderate: 0 | 1 | |
| | Severe: 0 | 1 | |
| | Total: 1 | 3 | |
| Headache (No.) | Mild: 5 | 1 | 0.083[b] |
| | Moderate: 1 | 1 | |
| | Severe: 0 | 4 | |
| | Total: 6 | 6 | |
| Diarrhea (No.) | Mild: 3 | 2 | 0.584[b] |
| | Moderate: 0 | 0 | |
| | Sever: 0 | 0 | |
| | Total: 3 | 2 | |
| Nausea (No.) | Mild: 1 | 4 | 0.168[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 1 | 4 | |
| Vomiting (No.) | Mild: 0 | 0 | |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 0 | 0 | |
| Abdominal Pain (No.) | Mild: 4 | 5 | 0.571[b] |
| | Moderate: 0 | 1 | |
| | Severe: 0 | 0 | |
| | Total: 4 | 6 | |
| Loss of taste (No.) | Mild: 4 | 4 | 0.57[b] |
| | Moderate: 1 | 0 | |
| | Severe: 0 | 1 | |
| | Total: 5 | 5 | |
| Loss of smell (No.) | Mild: 3 | 6 | 0.717[b] |
| | Moderate: 2 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 6 | 8 | |
| Rhinorrhea (No.) | Mild: 3 | 0 | 0.063[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 3 | 0 | |
| Sore throat (No.) | Mild: 0 | 4 | 0.078[b] |
| | Moderate: 1 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 1 | 4 | |
| Sweating (No.) | Mild: 1 | 6 | 0.237[b] |
| | Moderate: 2 | 1 | |
| | Severe: 1 | 1 | |
| | Total: 4 | 8 | |
| Anorexia (No.) | Mild: 3 | 7 | 0.286[b] |
| | Moderate: 2 | 3 | |
| | Severe: 0 | 0 | |
| | Total: 5 | 10 | |
| Chill (No.) | Mild: 1 | 3 | 0.316[b] |
| | Moderate: 0 | 0 | |
| | Severe: 0 | 0 | |
| | Total: 1 | 3 | |

$SpO_2$: saturation of peripheral oxygen, No.: number of patients, CRP: C-reactive protein, WBCs: White blood cells.
[a] Independent samples t-test, [b] Chi-squared test.

In this clinical trial, patients were questioned about different clinical symptoms and their severity according to the related criteria at the beginning and on days 3, 7, and 14 after initiating therapy (Tables 1-4). Patients in the minocycline group showed improvement in clinical symptoms at 6.85 ± 3.57 days after beginning the therapy compared to the placebo group (10.95 ± 5.16, days). According to the data, minocycline was able to reduce illness and increase life quality in COVID-19 patients.

Treatment of viral diseases is always challenging. One of the best ways to overcome viral infections is the modulation of the immune system.[25] In COVID-19 disease, abnormal leukocyte count has been observed, leading to the immune system suppression.[26] This condition is a viral escape mechanism for its replication in human cells.[27] Minocycline 100 mg twice daily resulted in WBC count normalization, which could likely modulate the immune system and reduce viral loads and disease contagiousness.

**Table 4.** Clinical characteristics in patients treated with minocycline and placebo group (day 14)

| | Placebo group (n = 20) | Treatment group (n = 20) | *P* value |
|---|---|---|---|
| Fever (°C) | 35.882 ± 0.40 | 35.165 ± 0.41 | 0.223[a] |
| SpO2 (%) | 96.00 ± 0.30 | 95.40 ± 0.60 | 0.384[a] |
| CRP (mg/L) | 3.31 ± 0.31 | 4.21 ± 0.42 | 0.095[a] |
| WBC (103/ul) | 5.69 ± 0.38 | 6.31 ± 0.32 | 0.224[a] |
| Lymphocytes/ul | 2251 ± 174 | 2311 ± 120 | 0.78[a] |
| Cough (No.) | Mild:1 | 6 | 0.052[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 6 | |
| Dyspnea (No.) | Mild:1 | 0 | 0.558[b] |
| | Moderate:1 | 1 | |
| | Severe:0 | 0 | |
| | Total:2 | 1 | |
| Malaise (No.) | Mild:3 | 7 | 0.392[b] |
| | Moderate:2 | 1 | |
| | Severe:0 | 0 | |
| | Total:5 | 8 | |
| Chest pain (No.) | Mild:0 | 3 | 0.142[b] |
| | Moderate:1 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 3 | |
| Body pain (No.) | Mild:1 | 1 | 0.935[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 1 | |
| Headache (No.) | Mild:5 | 2 | 0.114[b] |
| | Moderate:0 | 3 | |
| | Severe:0 | 0 | |
| | Total:5 | 5 | |
| Diarrhea (No.) | Mild:0 | 1 | 0.337[b] |
| | Moderate:0 | 0 | |
| | Sever:0 | 0 | |
| | Total:0 | 1 | |
| Nausea (No.) | Mild: 1 | 0 | 0.284[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 0 | |
| Vomiting (No.) | Mild:1 | 0 | 0.284[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 0 | |
| Abdominal Pain (No.) | Mild:1 | 2 | 0.509[b] |
| | Moderate:0 | 0 | |
| | Severe:1 | 0 | |
| | Total:2 | 2 | |
| Loss of taste (No.) | Mild:1 | 3 | 0.382[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 1 | |
| | Total:1 | 4 | |
| Loss of smell (No.) | Mild:2 | 4 | 0.660[b] |
| | Moderate:0 | 1 | |
| | Severe:1 | 1 | |
| | Total: 3 | 6 | |
| Rhinorrhea (No.) | Mild:1 | 0 | 0.284[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:1 | 0 | |
| Sore throat (No.) | Mild:2 | 1 | 0.481[b] |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:2 | 1 | |
| Sweating (No.) | Mild:1 | 4 | 0.381b |
| | Moderate:1 | 2 | |
| | Severe:1 | 0 | |
| | Total:3 | 6 | |
| Anorexia (No.) | Mild:0 | 2 | 0.134b |
| | Moderate:0 | 2 | |
| | Severe:0 | 0 | |
| | Total:0 | 4 | |
| Chill (No.) | Mild:0 | 0 | |
| | Moderate:0 | 0 | |
| | Severe:0 | 0 | |
| | Total:0 | 0 | |

$SpO_2$: saturation of peripheral oxygen, No.: number of patients, CRP: C-reactive protein, WBCs: White blood cells.
[a] Independent samples t-test, [b] Chi-squared test.

**Table 5.** Effect of minocycline on time interval until clinical symptoms improvement and time interval until WBCs, CRP and SpO2 reaching normal limits

| | Placebo group | Minocycline group | *P* value |
|---|---|---|---|
| Time interval until clinical symptoms improvement | 10.95 ± 1.18, day | 6.85 ± 0.79, day | 0.006 |
| Time interval until leukocytes reaching to normal limits | 6.72 ± 1.25, day | 3.95 ± 0.59, day | 0.046 |
| Time interval until CRP reaching to normal limits | 7.16 ± 1.17, day | 6.05 ± 1.00, day | 0.476 |
| Time interval until SpO2 reaching to normal limits | 5.39 ± 2.04, day | 1.90 ± 0.85, day | 0.112 |

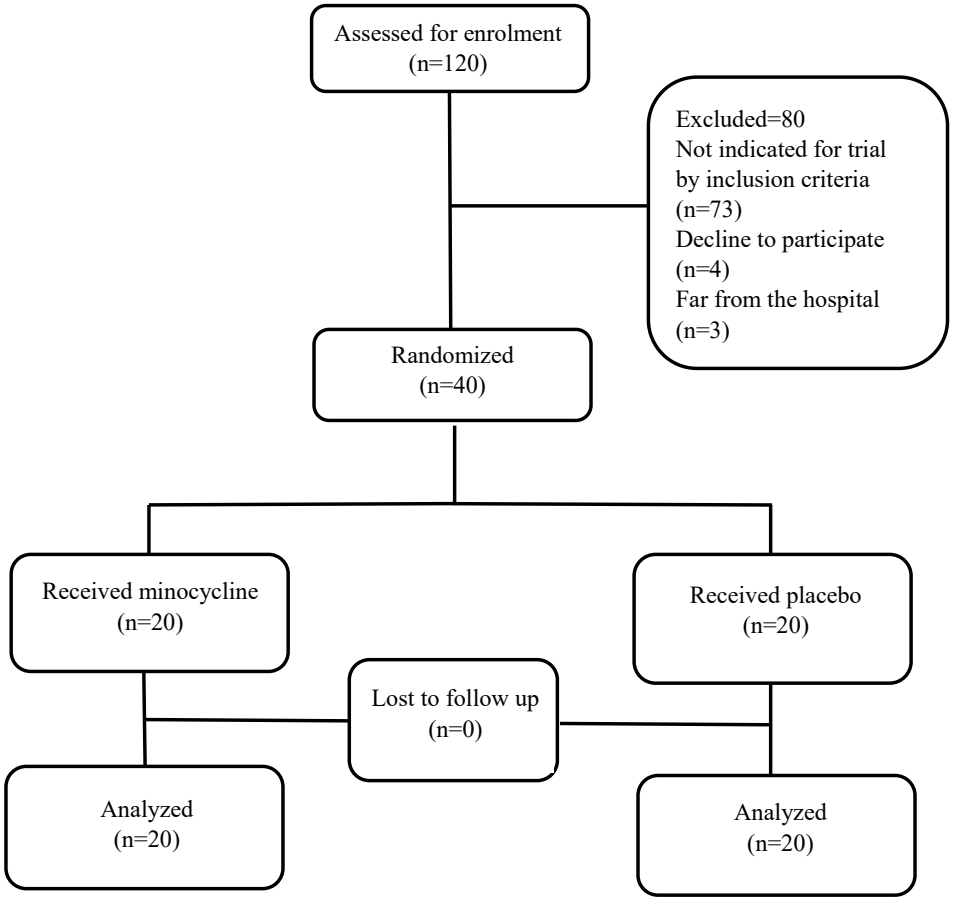


**Figure 1.** Patient enrollment and treatment assignment

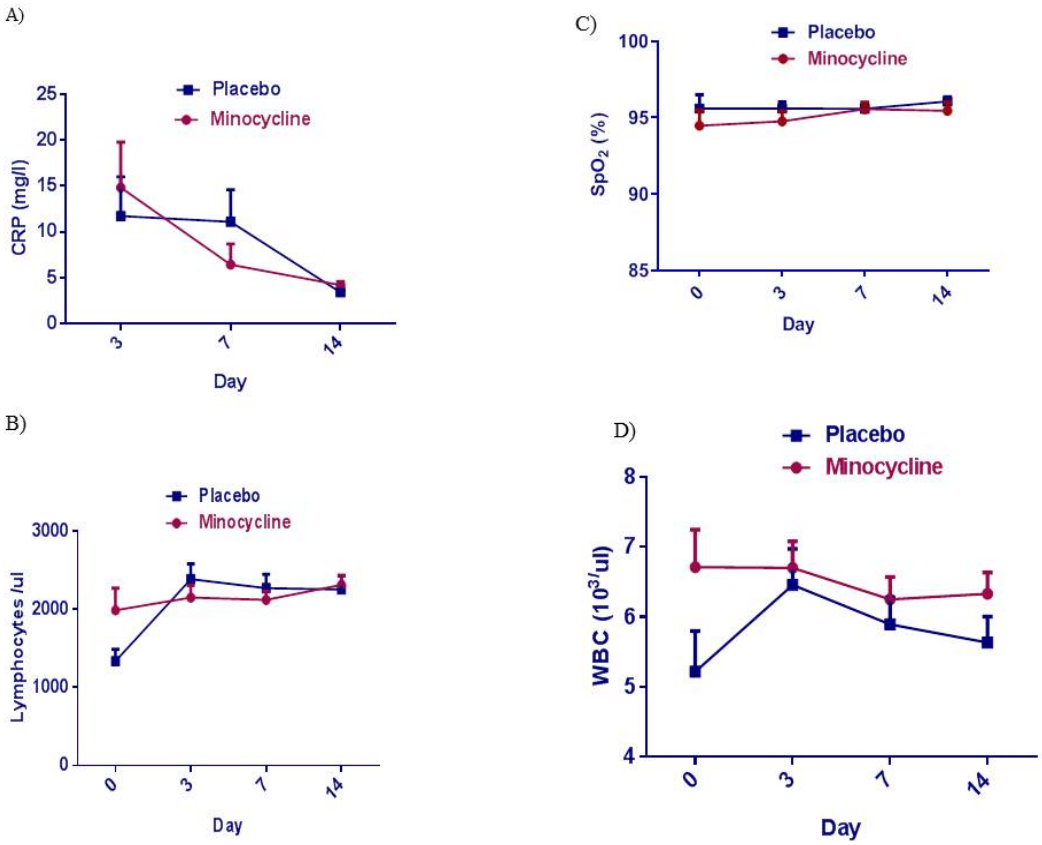


**Figure 2.** Effect of minocycline on CRP (A), lymphocytes (B), SpO2 (C), and WBC (D) levels in outpatients with COVID-19 at days 0, 3, 7, and 14. Repeated measures ANOVA

Immunomodulatory effects of minocycline have been reported in previous studies. Minocycline alters T-cell activation and decreased monocyte/macrophage activation in a simian immunodeficiency virus macaque model of HIV leading to neuroprotection. Moreover, minocycline reduced the viral load in the cerebrospinal fluid and plasma and decreased the infiltration of cytotoxic lymphocytes into the brain.[28] In another study the efficacy of minocycline combined with favipiravir in the management of COVID-19 patients was shown. The time interval between the beginning of treatment and COVID-19 PCR negative results was significantly reduced in the treatment group. Moreover, IL-6 and IL-8 were reduced in the treatment group. No differences in adverse effects were observed between the control and treatment groups.[29]

CRP is an inflammatory marker that is suggested to be measured in COVID-19 patients. This marker is produced by IL-6 in the liver. It is considered an "acute phase reactant".[30] CRP level is increased in COVID-19 disease. The severity of the disease depends on the CRP level.[31] In this study, CRP was positive both in the placebo and treatment groups at the beginning of the study. After seven days, CRP was non-significantly reduced in the minocycline group compared to the placebo group. This reduction has continued until 14 days (Figure 2A).

In this study, the Iranian National COVID-19 Therapy Regimen at the time were lopinavir-ritonavir or atazanavir-ritonavir, hydroxychloroquine, antipyretics, and H2 blockers. Patients received in both groups atazanavir/ritonavir (300/100 once a day for 5-7 days) or lopinavir/ritonavir (400/100 mg, BID for 5-7 days), hydroxychloroquine (two tablets, BID for the first day, then, one tablet, BID for 7 days), naproxen 500 mg, BID for 5 days and famotidine 40 mg, once a day for 10 days. Minocycline may exhibit synergistic effects with the above-mentioned antiviral and anti-inflammatory drugs. According to documents, minocycline is a potent anti-inflammatory agent.

Several mechanisms including inhibition of some enzymes activities such as phospholipase A2, inducible nitric oxide synthase, and matrix metalloproteinases, lowering protein tyrosine nitration, antiapoptotic effects by inhibiting caspase activation and increasing B-cell lymphoma 2, attenuating p38 mitogen-activated protein kinase phosphorylation, and inhibitory effects on poly (ADP-ribose) polymerase-1 activity may be involved in anti-inflammatory, immunomodulatory and neuroprotective effects of minocycline.[16]

Moreover, minocycline possesses antiviral effects in both *in vitro* and *in vivo* studies. Its antiviral effects against HIV have been shown. Minocycline attenuates HIV infection by reducing CCR5 expression and interfering with the HIV integrase enzyme that is necessary for entering and replication of HIV in cells.[18] Also, the efficiency of minocycline against influenza, rabies, HTLV1, reovirus, Japanese encephalitis, and West Nile has been proved during *in vitro* studies.[19] Moreover, it has protective effects and reduces cell damage against the RSV.[20] Minocycline exhibits anti-viral and anti-inflammatory effects against EV71 (entrovirus71) in both *in vivo* and *in vitro* studies. Minocycline (100-300 mcg/mL) diminished the expression of viral proteins, viral titer, cell damage, and levels of IL-8, IL6, IL12, P40 mRNA, IL1β, and TNF-α after EV71 infection. TNF-α, IL-6, IL-8, and IL1-β levels were decreased with minocycline single-dose administration in THP-1 (a human monocytic leukemia cell line) infected cells by EV71. Viral titer, mortality rate, and clinical scores were reduced by minocycline. This drug inhibited IL-6 as well as granulocyte colony-stimulating factor in plasma and TNF-α in the cerebellum.[32]

Additionally, in a randomized double-blind placebo-controlled study conducted on HIV patients with cognitive dysfunction, there is no significant difference in cognition performance between minocycline (100 mg, BID for 24 weeks) and placebo groups.

Minocycline could reduce clinical symptoms interval improvement due to its anti-inflammatory and antiviral effects. So, it may be suggested that minocycline is effective in both inflammatory and viral replication phases of the COVID-19 disease.

Considering that inpatients have not been evaluated in our study, thus, the effect of minocycline on severe COVID-19 disease or mortality rate has not been investigated. According to the results of this study, it could be suggested that minocycline as a promising agent can be used in further investigations to evaluate its effect on mortality rate and hospitalization.

## Conclusion

In this randomized double-blind placebo-controlled study, minocycline (100 mg, BID for 14 days) reduced the duration of clinical symptoms improvement as well as the duration of WBC normalizing in outpatients of COVID-19 disease.

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**Authors' Contribution**

**Conceptualization:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor.
**Data curation:** Danial Esmaeilzadeh, Mohammad Shariati Rad.
**Formal analysis:** Danial Esmaeilzadeh, Mohammad Shariati Rad.
**Funding acquisition:** Hossein Hosseinzadeh.
**Investigation:** Danial Esmaeilzadeh, Mohammad Shariati Rad, Bibi Marjan Razavi.
**Methodology:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor.
**Project administration:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor.
**Resources:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor
**Software:** Danial Esmaeilzadeh, Mohammad Shariati Rad.
**Supervision:** Hossein Hosseinzadeh, Amir Hooshang

## Study Highlights

***What is current knowledge?***

- Minocycline a semi-synthetic tetracycline is a therapeutic option for COVID-19 because of its anti-inflammatory and immunomodulatory effects.

***What is new here?***

- Minocycline reduced the duration of clinical symptoms improvement as well as the duration of white blood cell (WBC) normalizing in outpatients of COVID-19 disease.

Mohamadpoor, Bibi Marjan Razavi, Mehrdad Esmaeilzadeh.
**Validation:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor.
**Visualization:** Danial Esmaeilzadeh, Mohammad Shariati Rad.
**Writing–original draft:** Danial Esmaeilzadeh, Mohammad Shariati Rad.
**Writing–review & editing:** Hossein Hosseinzadeh, Amir Hooshang Mohamadpoor.

### Competing Interests

The authors declared no conflicts of interest

### Ethical Approval

The protocol was approved by the Iranian Committee of Ethics in Human Research (Registration number: IR.MUMS.REC.1399.053) on April 6, 2020. The trial was also registered at Iranian Registry of Clinical Trials (identifier: IRCT20081019001369N4).

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