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Coronavirus Pandemic

Effectiveness of levamisole in the treatment of patients with severe COVID-19: a randomized controlled clinical trial

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Abstract

Introduction: Inflammation plays a role in coronavirus disease 2019 (COVID-19) pathophysiology and anti-inflammatory drugs may help reduce the disease severity. Levamisole is an anthelmintic drug with immunomodulatory and possible antiviral effects. This study aimed to evaluate the role of levamisole in the treatment of patients with COVID-19.

Methodology: In this randomized controlled clinical trial, hospitalized patients with confirmed severe COVID-19 (arterial oxygen saturation < 90%) were randomly assigned to either experimental (n = 41) or control (n = 45) groups. Levamisole 50 mg orally daily was prescribed for the first group until discharge or death, for a maximum of 7 days, along with other standard treatments. Only standard treatments were prescribed to the control group. Clinical response (either improvement or failure) at the end of the fifth and seventh days, changes in laboratory indices, length of hospitalization, and mortality rate during the study were evaluated and recorded.

Results: The rate of clinical improvement in the experimental group was significantly more than in the control group on the fifth (97.6% vs. 58.7%, $p < 0.001$) and seventh (95.8% vs. 66.7%, $p = 0.007$) days. Furthermore, the mean length of hospital stays in the experimental group (8.39 ± 3.54 days) was significantly shorter than in the control group (10.78 ± 5.40 days, $p = 0.024$). No patients died during the study.

Conclusions: Administering levamisole to hospitalized patients with severe COVID-19 reduced hospitalization time and improved several clinical outcomes.

Key words: Levamisole; COVID-19; inflammation; immune response; clinical trial.

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Introduction

The virus that causes coronavirus disease 2019 (COVID-19) was discovered in December 2019 in Wuhan, China, and the World Health Organization (WHO) declared the outbreak an international concern on 30 January 2020. COVID-19 was declared a pandemic in March 2020 [1], and has caused over 6 million deaths worldwide [2]. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the primary pathogen of COVID-19 and can lead to severe lung infection.

WHO determined that the average death rate from COVID-19 is 0.27% based on the data from 51 locations. Although the death rate is low, this disease is highly contagious. The prevalence and death rates in Iran are 34.6% and 11.1%, respectively [1,3].

Since this was a new disease, different treatment protocols and methods were proposed. The use of drugs with viral enzyme (e.g., RNA polymerase) inhibition mechanisms; and anti-inflammatory drugs, including corticosteroid drugs, were among the most important therapeutic methods used to control the disease [4,5].

Based on the available data, it appears that infections caused by this virus cause a severe immune response in the host. As a result, the plasma concentration of inflammatory cytokines is significantly elevated in patients with COVID-19, leading to an event known as cytokine storm [6]. Excessive inflammatory responses occur in this disease, causing lung tissue damage. Unfortunately, in some cases, the inflammation causes a significant drop in blood oxygen saturation, which can lead to death [7].

Given the role of inflammation in COVID-19 pathophysiology, anti-inflammatory drugs may be used as a supportive treatment to help modulate immunity and reduce disease severity. The severity of the COVID-19 is directly related to the occurrence of cytokine storms [8]. In general, corticosteroids can reduce inflammatory responses and acute lung injury. During viral infection outbreaks, corticosteroids suppress the cytokine storm and immune responses. However, using these drugs may result in side effects such as an increased risk of secondary infection and delayed viral clearance [9].

Levamisole (LMS) is a well-known anthelmintic drug with strong immunostimulatory properties. It is a synthetic phenylimidazothiazole. This drug can boost cellular immunity depending on the dose and timing of administration [10]. Levamisole is used as an immune system stimulant in bacterial or viral infectious diseases, rheumatoid arthritis, and in treating malignant diseases such as colon cancer [11]. This drug enhances T cell function by modulating cellular immune responses [12,13]. Levamisole improves cellular and humoral immunity by modulating pro-inflammatory cytokines like interleukin-6 and interleukin-8 [14–16].

Since COVID-19 is a new disease, various protocols and treatment methods have been proposed and used for its management. Immunomodulator drugs are one of these treatments [6] with levamisole being one of the drugs that works by inducing a type 1 immune response. It has strong immunostimulatory effects through modulating both cellular and humoral immune responses. This effect has been suggested to be beneficial in the early stages of COVID-19, as it can aid in immune stimulation, SARS-CoV-2 clearance, and tissue repair [17,18]. However, levamisole has received little attention as a treatment for COVID-19 patients. Considering the pathophysiology of COVID-19 and the immunomodulating properties of levamisole, this clinical trial was designed to assess levamisole effectiveness in treating COVID-19.

Methodology

This study was a randomized controlled clinical trial performed at Al-Zahra Hospital, Isfahan, Iran, affiliated with the Isfahan University of Medical Sciences (IUMS), from November 2020 to May 2021. The study protocol was registered in Iranian Registry of Clinical Trials (IRCT) under the code IRCT20181208041886N1.

Study population

The study population consisted of patients hospitalized with severe COVID-19. The patients were chosen using convenience sampling. The inclusion criteria were: age 18 years or older, COVID-19 diagnosis by reverse transcriptase polymerase chain reaction (RT-PCR) test and/or a chest computed tomography (CT) scan reviewed by an infectious disease specialist, arterial oxygen saturation (SpO_2) less than 90%, and hospitalization. It should be noted that according to the Iranian guideline of COVID-19 diagnosis and treatment published by Iran's Ministry of Health and Medical Education [19], the criteria for severe disease included: 1) rapid progression of respiratory symptoms especially dyspnea; 2) tachypnea (respiratory rate > 30/min); 3) $SpO_2 < 90\%$; and 4) lung involvement > 50% in CT scan. However, in this study, only the third criterion was considered for the inclusion of patients.

The exclusion criteria included: presence of another cause of the patient's symptoms (such as a bacterial or fungal infection), hospitalization in the intensive care unit (ICU) or meeting its criteria, a history of allergic reaction to levamisole, taking any antibacterial agent, pregnancy, breastfeeding, home oxygen therapy, underlying lung disease, malignancy, glucose-6-phosphate dehydrogenase (G6PD) deficiency; underlying immunodeficiency, and/or use of immunosuppressive drugs.

Ethical considerations

All patient data were kept safe and confidential under the responsibility and strict supervision of the research executives. The study objectives were explained to the patients before enrollment, and informed consent was obtained. In addition, the patients were assured that their treatment would not be disrupted if they did not participate in the study. The ethics committee of IUMS approved the study with the ethics code IR.MUI.MED.REC.1399.246.

Sample size

A sample size of at least 50 participants in each group was considered for this study due to the absence of comparable published clinical trials.

Interventions

The selected patients were randomly assigned to either experimental or control groups. The randomization was done via block randomization method with blocks of four. All states of quadruple

arrangement (two intervention cases and two control cases in each block) were designed. The patients were assigned to one of two groups according to order in the selected block. All demographic and clinical data, including age, gender, underlying disease, consumed drugs, and several blood tests were recorded before hospitalization.

Levamisole (Poursina, Tehran, Iran) tablets, 50 mg taken orally daily, were prescribed for patients in the test group until discharge or death, and for a maximum of 7 days, along with other necessary standard treatments. Only standard treatments were prescribed to patients in the control group. Remdesivir, (Actover, Karaj, Iran) 200 mg on the first day, and 100 mg intravenous infusion daily from the second to the fifth day; and dexamethasone (Iran Hormone, Tehran, Iran) 8 mg intravenous daily, were the standard treatments. All patients in both groups received the necessary symptomatic and supportive treatments, including oxygen therapy, thromboembolism prophylaxis, antitussive treatment (dextromethorphan), and stress ulcer prophylaxis (famotidine). Throughout the study, the clinical status, including fever reduction, improvement in shortness of breath, cough reduction, blood oxygen saturation (SpO_2), and hemodynamic parameters, were monitored and controlled daily.

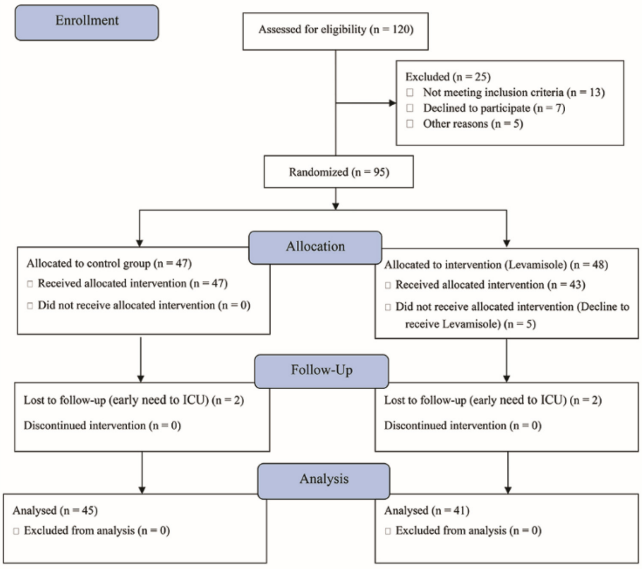
If the patient received the drug for less than three days, developed a secondary bacterial or fungal infection, suffered from thromboembolism (pulmonary embolism, deep vein thrombosis), or required intensive care and was admitted to the ICU, he/she was excluded from the study.

Outcome variables

The primary outcome was clinical response at the end of the fifth and seventh days of hospitalization, and was defined as either improvement (body temperature < 37.8 °C, cough reduction, respiratory rate < 20/min, and SpO_2 > 93% in room air) or failure (continued or worsening of the patient's initial symptoms). This judgment was made by the physician. Changes in laboratory indices and vital signs, length of hospitalization, need for intensive care, discharge rate during the intervention period, and mortality rate during the study were the secondary outcome variables.

Patient discharge criteria, based on national guidelines; included a fever-free period of 48–72 hours without antipyretics; SpO_2 > 93% or > 90% in the previous two to three days in room air; and improvement in clinical, respiratory, and vital signs. The decision for discharge was made by the physician.

Figure 1. The CONSORT flowchart of the study.



ICU: intensive care unit.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 24 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The Chi-square test was used to compare categorical baseline demographic and clinical characteristics, the clinical response, and mortality rate between the two groups. Independent samples t-test was used to compare blood tests and the length of hospitalization between the two groups. Repeated measures analysis of variance (ANOVA) was used for comparison of the values in the evaluated times within each group. $p < 0.5$ was considered significant.

Results

Patients

Figure 1 depicts the study's consolidated standards of reporting trials (CONSORT) flowchart. During the study, 97 patients were randomly assigned to the two groups with four people in the experimental group and seven in the control group being excluded from the study due to the short duration of participation.

The primary demographic and clinical characteristics of the patients studied are shown in Table 1. There was no significant difference in any of the basic variables between the two groups.

Outcome variables: variations in laboratory parameters

Table 2 summarizes the changes in laboratory indicators of the studied patients. There was no significant difference between the two groups in any of the mentioned parameters.

Table 1. Baseline demographic and clinical characteristics of study patients.

Parameter	Group		*p* value
	Levamisole (n = 41)	Control (n = 45)	
Age (years)	57.14 ± 15.15 (26–83)	61.2 ± 17 (21–89)	0.248
White blood cells (cells/mm^3)	7930 ± 3375.11 (3700–14500)	9886.66 ± 6746.19 (2000–37300)	0.395
Neutrophils (cells/mm^3)	80.78 ± 8.31 (61.1–94.9)	77.1 ± 11.1 (47.1–95)	0.091
Lymphocytes (cells/mm^3)	13.06 ± 6.8 (3.1–26.6)	14.85 ± 8.8 (3.5–40.8)	0.297
Platelets (cells/mm^3)	178814.6 ± 67062.04 (14100–322000)	201717.8 ± 95932 (9300–619000)	0.241
SpO_2 without oxygenation (%)	87.58 ± 4.87 (75–95)	86.36 ± 7.79 (52–98)	0.701
SpO_2 with oxygenation (%)	94.75 ± 3.03 (88–99)	95.28 ± 3.64 (80–99)	0.240
CRP (mg/L)	78 ± 33.37 (7–132)	67.24 ± 40.27 (3–131)	0.213
ESR (mm/h)	49.3 ± 26.31 (3–120)	47.26 ± 30 (4–111)	0.581
SCr (mg/dL)	1.11 ± 0.22 (0.8–1.8)	1.59 ± 1.7 (0.6–11.5)	0.287
ALT (U/L)	52.67 ± 31.63 (14–160)	54.8 ± 81.51 (13–429)	0.878
AST (U/L)	46.25 ± 17.94 (23–114)	52.51 ± 47.32 (18–285)	0.245
Temperature (°C)	37.44 ± 0.58 (36.5–38.7)	37.68 ± 0.98 (36–40)	0.247
RR (breaths/min)	20.36 ± 3.69 (15–29)	23.42 ± 10.82 (16–74)	0.355
HR (beats/min)	90.95 ± 18.97 (60–139)	95.28 ± 15.21 (70–125)	0.363
Comorbidity			
HTN	5 (12.2)	3 (6.5)	
DM	2 (4.9)	2 (4.3)	
HTN+DM	2 (4.9)	3 (6.5)	
HLP	2 (4.9)	–	0.247
HTN+HLP	2 (4.9)	–	
HTN+DM+ HLP+IHD	1 (2.4)	2 (4.3)	
HLP+DM+HTN	1 (2.4)	3 (6.5)	
IHD	2 (4.9)	1 (2.2)	
Hypothyroidism	1 (2.4)	-	

ALT: alanine transaminase; AST: aspartate transaminase; CRP: C-reactive protein; DM: diabetes mellitus; ESR: erythrocyte sedimentation rate; HLP: hyperlipidemia; HR: heart rate; HTN: hypertension; IHD: ischemic heart disease; RR: respiratory rate; SCr: serum creatinine; SpO_2: oxygen saturation.

Other outcome variables

Table 3 lists the variables investigated in this study. The rate of clinical improvement on the fifth and seventh days in the experimental group (levamisole) was significantly more than in the control group. Furthermore, the mean length of stay in the test group (levamisole) was significantly shorter than in the control group. The need for intensive care and the discharge rate in the test group were lower and higher, respectively, in the experimental group compared to control, and these differences were not statistically significant.

It should be noted that no patients died during the study.

Discussion

This study investigated the effectiveness of levamisole in combination with standard treatment in improving the clinical status of hospitalized patients with COVID-19. The drug accelerated patients'

Table 2. Changes in laboratory parameters of the study patients.

Parameter	Time (day)	Group		*p* value
		Levamisole (n = 41)	Control (n = 45)	
WBC (Cells/mm^3)	0	7930 ± 3375.11 (3700–14500)	9886.66 ± 6746.19 (2000–37300)	0.395
	5	9895 ± 3511.88 (4600–21100)	9535.29 ± 5499.08 (2100–25700)	0.283
	7	10410.53 ± 3894.56 (4400–17000)	15728.57 ± 26068.9 (2600–146000)	0.888
p value		0.095	0.356	0.599
Neutrophils	0	80.78 ± 8.31 (61.1–94.9)	77.1 ± 11.1 (47.1–95)	0.144
(cells/mm^3)	5	82.43 ± 7.72 (59.9–95.5)	77.57 ± 12.81 (49–99)	0.101
	7	81.36 ± 7.55 (68.6–92.3)	73.94 ± 15.21 (28.2–96.1)	0.104
p value		0.276	0.354	
Lymphocytes	0	13.06 ± 6.8 (3.1–26.6)	14.85 ± 8.8 (3.5–40.8)	0.486
(Cells/mm^3)	5	10.62 ± 5.91 (2.3–31.8)	15.55 ± 11.72 (0.7–59)	0.089
	7	11.39 ± 6.56 (2.9–26.1)	14.95 ± 9.79 (2.1–44.4)	0.206
p value		0.358	0.803	
Platelets	0	178814.6 ± 67062.04 (14100–322000)	201717.8 ± 95932 (9300–619000)	0.241
(Cells/mm^3)	5	244642.5 ± 86780.27 (26700–550000)	263212.1 ± 479429.5 (54000–2880000)	0.004
	7	241635 ± 79961.64 (26700–337000)	205692.3 ± 105969.3 (55000–531000)	0.04
p value		< 0.001	0.541	
SpO_2 without	0	87.58 ± 4.87 (75–95)	86.36 ± 7.79 (52–98)	0.701
oxygenation (%)	5	88.65 ± 6.32 (67–96)	85.68 ± 9.42 (45–98)	0.118
	7	87.95 ± 7.51 (69–99)	86.36 ± 7.28 (67–98)	0.271
p value		0.349	0.598	

SpO_2: oxygen saturation; WBC: white blood cell.

Table 3. Results of evaluated outcome variables in study patients.

Parameter	Group		*p* value
	Levamisole (n = 41)	Control (n = 45)	
Duration of hospitalization (mean ± SD)	8.39 ± 3.54 (4–20)	10.78 ± 5.40 (5–30)	0.024
Clinical response on day 5 (n, %)			< 0.001
Improvement	40 (97.6 %)	27 (58.7 %)	OR:1.662
Failure	1 (2.4 %)	19 (41.3 %)	(1.298–2.128)
Clinical response on day 7 (n, %)			0.007
Improvement	23 (95.8)	24 (66.7)	OR:1.438
Failure	1 (4.2)	12 (33.3)	(1.124–1.838)
Need to ICU care (n, %)	4 (9.8 %)	6 (13 %)	0.743
Discharge during intervention (n, %)	19 (46.3 %)	13 (28.3 %)	0.081
			OR: 1.640
			(0.931–2.888)

ICU: intensive care unit; SD: standard deviation.

recovery and reduced the length of their hospitalization, but it did not affect the need for intensive care (intubation).

There is only one published clinical study available on the effect of levamisole in the treatment of COVID-19, which was performed on outpatients with mild to moderate disease (SpO_2 > 94%). In a study conducted by Roostaei Firozabad *et al*., administration of 50 mg levamisole three times a day for three days was associated with a reduction in cough on the third and fourteenth days of the intervention and an improvement in shortness of breath on the seventh and fourteenth days, in comparison to the placebo. Other symptoms (such as fever, headache, and weakness) showed no significant differences compared to the placebo [20]. This study differed from our work in several aspects including the studied population (in terms of disease severity), and the dose and duration of levamisole use. However, both studies show that levamisole positively affects some parameters of patient recovery.

The modulating effects of levamisole on the body's immunity, such as modulating interferons, interleukin-6, and interleukin-8, may be the mechanism by which levamisole has a beneficial effect in COVID-19 [17,18]. Levamisole improves T cell and B cell functions, and antibody production, while decreasing immune complexes [21]. Levamisole is associated with increase in interferon-gamma (IFN-γ), CD4/CD8 ratio, and IL-18. It is also linked to a decrease in IL-4 [14]. In allergic rhinitis models, interleukins 4, 5, and 13 were also reduced [22]. The significant reduction in C reactive protein (CRP) observed in the test group (levamisole) may be due to the same effect of levamisole.

COVID-19 appears to have three phases: the initial infection phase, the pulmonary phase, and the severe inflammatory phase [23]. The first phase (5 to 7 days after onset of symptoms) is caused by the virus directly, and the subsequent two phases are caused by the body's inflammatory response (7 to 15 days from the onset of symptoms). As a result, anti-inflammatory drugs appear to help alleviate the effects of this disease [24]. Since many patients visit doctors and medical centers after a few days of the onset and intensification of symptoms, most of the participants possibly were in the inflammatory phase. Hence, the anti-inflammatory effects of levamisole played a role in improving the patients' condition.

On the other hand, the possible antiviral effect of levamisole may also be involved in the positive effects observed in this study. The anti-rotavirus effect of levamisole through increasing the release of IFN-γ and IgG has been reported [25]. Levamisole has also been shown to improve the humoral immune response to the hepatitis B vaccine and the human immunodeficiency virus (HIV) [26]. A recent study reported that levamisole has the potential to improve the humoral immune response in HIV-infected patients by increasing the expression of IL-4 [13]. However, more research is needed to determine the effect of levamisole on SARS-CoV-2. This medication may prevent SARS-CoV-2 replication by inhibiting the virus's papain-like protease enzyme (PL-pro) [14]. This enzyme is essential for breaking the virus polyproteins to release non-structural proteins, glycation of the virus polyproteins, and virus multiplication, as well as inhibiting the host's innate immunity [14,27]. In silico studies have shown that levamisole is one of the drugs that may be able to inhibit the SARS-CoV-2 virus PL-pro [27]. Furthermore, the effect of levamisole on increasing the expression and signaling of glucocorticoid receptors, as demonstrated by the drug's efficacy in corticosteroid-sensitive nephrotic syndrome [28], may be involved [29]. This is consistent with the findings of our study, since all patients received corticosteroid (dexamethasone) as part of the standard treatment, and its effects might have been enhanced by levamisole.

Nonetheless, one of the theoretical concerns with using levamisole in COVID-19 is the possibility of

exacerbating the cytokine storm due to its immune-enhancing effects. In theory, increasing Th1 activity and subsequent rise of inflammatory cytokines on one hand, and decreasing Th2 activity (which has an anti-inflammatory effect) on the other, can result in such a destructive effect. As a result, it appears that the timing of levamisole administration in COVID-19 influences its outcome. Prospective clinical studies are required to determine the efficacy of this drug and the best time to administer it in COVID-19.

Considering the findings of Roostaei Firozabad *et al.* [20] and the current study, levamisole can potentially improve some clinical symptoms of COVID-19. However, some important indicators of clinical recovery in this disease, such as oxygenation status (SpO_2), did not show a significant difference compared to the control group. Hence, administering levamisole with this regimen (50 mg per day for seven days) may be accompanied by minor positive effects. As previously stated, additional studies with larger sample sizes and higher doses are required to confirm or reject the effects of this drug on COVID-19.

Conclusions

Administering levamisole to hospitalized patients with severe COVID-19 reduced hospitalization time and improved several clinical outcomes, but it did not reduce the need for intensive care.

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Authors' contributions

MP: study idea, patient selection, data interpretation; FK: patient selection, data interpretation; BA: patient selection, data interpretation; RS: study design and protocol, data analysis and interpretation, manuscript draft; MHN, MS, FN: data collection and recording; All authors reviewed the final version of manuscript.

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