ORIGINAL ARTICLE **DOI: https://doi.org/10.18502/fem.v6i3.9395**

Efficacy of levamisole with standard care treatment vs. standard care in clinical presentations of non-hospitalized patients with COVID-19: a randomized clinical trial

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Published online: 2022-03-02

Abstract: **Objective:** The aim of this study was to evaluate the influence of adding a 10-day course of levamisole (LVM) to the standard care compared with standard care alone, on the clinical status of COVID-19 patients with mild to moderate disease.

Methods: In this randomized open-label trial, we enrolled non-hospitalized patients with mild to moderate COVID-19 at nine health centers in Tehran province, Iran, in 2021. Patients were randomly assigned to receive a 10-day course of LVM with standard care (n=185) or standard care alone (n=180) in a 1:1 ratio. On days 1 to 10, LVM was administered orally at a dosage of 50 mg. The participants were called and followed on days 1, 3, 5, 7, 9, and 14. The measured parameters were general health condition, hospitalization rate, signs and symptoms, and adverse events. The generalized estimating equations model was used for analysis.

Results: Among 507 randomized patients, 473 patients started the experiment and received LVM plus standard care or received the standard care alone; 385 patients included in the analysis; 346 (98%) patients completed the trial. The median age of the patients was 40 years [IQR: 32-50.75]; and 201 (55.1%) patiens were male. The mean age, sex ratio, and frequency of the underlying diseases of the patients in the two study groups had no statistically significant differences (P>0.05). Compared to the control group, LVM improved the general health condition of the patients (B=-0.635; 95% CI: -0.041,-0.329; P<0.001). Patients receiving LVM compared with standard care group had significantly lower odds of developing fever (OR=0.260; 95% CI: 0.113,0.599; P=0.002), chills (OR=0.223; 95% CI: 0.076,0.648; P=0.006), fatigue (OR=0.576; 95% CI: 0.346,0.960; P=0.034), and myalgia (OR=0.544; 95% CI: 0.317,0.932; P=0.027). No significant difference was observed in the rate of hospitalization. Although the intervention group had greater adverse effects than the control group, the difference was not statistically significant.

Conclusion: Findings of this study suggest that LVM has clinical benefits in improving patients' health condition with mild to moderate COVID-19.

Keywords: COVID-19; Levamisole; Randomized Controlled Trial; SARS-CoV-2; Signs and Symptoms

Cite this article as: Asgardoon MH, Emadi Koochak H, Kazemi-Galougahi MH, Zare Dehnavi A, Khodaei B, Behkar A, et al. Efficacy of levamisole with standard care treatment vs. standard care in clinical presentations of non-hospitalized patients with COVID-19: a randomized clinical trial. Front Emerg Med. 2022;6(3):e33.

1. Introduction

In late 2019, a new strand of coronaviruses was discovered in Wuhan, China. This virus caused a disease named Coronavirus Disease 2019 (COVID-2019); Later, World Health Organization (WHO) announced the COVID-19 as a public health emergency of international concern (1). According to WHO statistics, the disease has infected millions of people worldwide and caused considerable number of deaths till now (2).

Almost no effective therapy exists for the outpatient treatment of COVID-19 and researchers are still working on it. Reducing symptom severity and decreasing hospitalization for outpatients are essential public health mitigation strategy for overcoming this pandemic (3).

Levamisole (LVM) is a safe, low-cost, widely available drug, that showed in vitro activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has been proposed as a potentially effective treatment (4-7). Several recent clinical trials highlighted that LVM might be effective in preventing and treating SARS-CoV-2 infections. Still, only one has been published, which was small in sample size and had methodological limitations, such as not removing the potential influence of time or the interactions (4). However, when taken early in the course of the disease, LVM may have therapeutic benefits in treating mild to moderate disease (6). To our knowledge, no randomized clinical trials to date have investigated LVM for COVID-19 in non-hospitalized patients with a large sample size. We hypothesized that starting LVM therapy during the first few days of symptoms' onset could alter the course of COVID-19 by reducing symptoms' severity and duration, as well as preventing hospitalization. Our objective was to evaluate the influence of adding a 10-day course of LVM to the standard care compared with standard care alone, on the clinical status of COVID-19 patients with mild to moderate disease.

2. Methods

2.1. Study design and setting

We conducted a 6-month, prospective, parallel, randomized, clinical trial on patients above 18 years old with mild to moderate COVID-19 who presented to 10 selected health centers of Tehran and its suburbs from mid-April 2021 to mid-September 2021. The Ethics Committee of AJA University of Medical Sciences (Ethics ID: IR.AJA.REC.1399.199) and the Iranian Registry of Clinical Trials both approved this study (ID: IRCT20201124049480N1, first registration date: 28/03/2021; The full protocol is available on https://en.irct.ir/trial/54675). Written informed consent was obtained from all patients or legally authorized representatives.

2.2. Study population

It was a multicenter study and convenience sampling was used in nine selected COVID-19 health centers. In order to selection of the COVID-19 health centers for this study, all centers in Tehran were assessed; we selected the centers qualified with 1) the infrastructures compatible with our study method, 2) the minimum number of work force required for implementing this study, and 3) the healthcare team who gave their consent to cooperate in this study. All patients who met the study inclusion criteria were referred to the selected centers from mid-April 2021 to mid-September 2021.

The inclusion criteria were as follows: patients aged 18 years or older with either signs and symptoms indicating COVID-19 or spiral chest computed tomography (CT) scan indicating COVID-19 or reverse transcription polymerase chain reaction (RT-PCR) confirming COVID-19 infection, and no use of LVM during the previous five days for any reason e.g., parasitic infection (due to the 16-hour half-life of the drug) who did not require hospitalization. We informed patients not to take medications that were not part of the study protocol since they were candidates for outpatient care.

Patients were excluded if they met the following criteria: negative RT-PCR for COVID-19 over the study period, history of hepatitis, cirrhosis, or severe liver disorders, severe renal failure (estimated glomerular filtration rate less than 30 mL/min), shortness of breath due to cardiogenic pulmonary edema, history of allergic reaction or known allergy or any hypersensitivity reaction to LVM, history of cancer chemotherapy, lactating or pregnant women or planning to become pregnant within 30 days of the trial.

COVID-19 was diagnosed based on the findings of the RT-PCR test (using the RT-PCR technique with Pishtazteb kit from the Pishtazteb company in Iran). However, due to the highest efficacy of outpatient management of COVID-19 (i.e., levamisole) in the first four days of symptoms onset, we sought to enroll patients as soon as possible. The median false-negative rate of RT-PCR testing was found to be 38% on the first day of symptom onset (range, 18-65%), decreasing during the subsequent days (8,9). We enrolled all suspected, probable and confirmed cases according to WHO definition (10), instead of waiting for RT-PCR results that may take up to 4 days to be available in our setting. The follow-up was continued unless the RT-PCR result was negative.

According to the national guideline for COVID-19 diagnosis and management, mild COVID-19 symptoms include fever<38°C, sore throat with or without dry cough, chills, headache, anosmia, dysgeusia, nausea, vomiting, anorexia, diarrhea, myalgia, and fatigue. Patients may present with one or more of the symptoms. The vital signs including pulse rate, blood pressure, and respiratory rate, are stable at this stage, and SpO2 (oxygen saturation level) is greater than 92%. The moderate disease is attributed to the patients suffering from more severe aforementioned symptoms besides respiratory symptoms (including shortness of breath, chest pain, and discomfort, etc.) with or without fever>38°C, and SpO2 ranged from 90% to 93% (11,12).

2.3. Sample size

Except for one clinical trial with 25 participants in each group, there is no complete study that can be used to calculate the sample size. However, considering the general health condition measured by verbal numeric scale (VNS) as the primary outcome, the significant level of α=0.05, power of 1-β=0.90, the minimum detectable difference between two groups *d=0.3*, the standard deviation (SD)=1.7, and attrition rate=10%, the required sample size was calculated as 180 pa-

Table 1 Comparison of baseline characteristics of the non-hospitalized patients with COVID-19 treated by levamisole and standard care

Characteristics	Control group (n=180)	Intervention group (n=185)	P
Gender, n (%)			
Male	91 (50.6)	110 (59.5)	0.087
Female	89 (40.4)	75 (40.5)	
Age (year), median (IQR)	41 (34-55)	37 (30-48)	0.056
<20, n (%)	3 (2.8)	6 (4.2)	-
20-29, n (%)	12 (11.1)	24 (16.8)	-
30-39, n (%)	28 (25.9)	45 (31.5)	-
40-49, n (%)	24 (22.2)	36 (25.2)	-
50-59, n (%)	29 (26.9)	17 (11.9)	-
60-69, n (%)	8 (7.4)	15 (10.5)	-
≥70, n (%)	4 (3.7)	0 (0.0)	-
Body mass index (kg/m^2), mean (SD)	24.4 (3.6)	25.2 (4.1)	0.137
<18.5, n (%)	2 (1.9)	4 (2.8)	-
18.5-24.9, n (%)	58 (55.2)	69 (47.6)	-
25-29.9, n (%)	39 (37.1)	58 (40.0)	-
≥30, n (%)	6 (5.7)	14 (9.7)	-
Vital signs			
Systolic blood pressure (mmHg), median (IQR)	120 (110-120)	120 (110-120)	0.425
Diastolic blood pressure (mmHg), median (IQR)	75 (70-80)	75 (70-80)	0.920
Pulse rate (beats/min), mean (SD)	80.4 (8.0)	82.4 (11.6)	0.104
Respiratory rate (breaths/min), median (IQR)	16 (15-18)	16 (14-18)	0.371
Temperature (°C), median (IQR)	37.0 (36.0-37.0)	37.0 (36.0-37.0)	0.721
Mean of room-air O2 Sat (%), median (IQR)	96 (95-97)	96 (95-97)	0.755
Social history, n (%)			
Cigarette smoking	7 (3.9)	7 (3.8)	0.958
Hookah smoking	3 (1.7)	5 (2.7)	0.499
Opium abuse	2 (1.1)	1 (0.5)	0.619
Co-existing conditions, n (%)			
Diabetes mellitus type I	1 (0.6)	1 (0.5)	1.000
Diabetes mellitus type II	19 (10.6)	13 (7.0)	0.227
Asthma	2 (1.1)	2 (1.1)	0.974
Chronic obstructive pulmonary disease	2 (1.1)	1 (0.5)	0.618
Hypertension	16 (8.9)	16 (8.6)	0.922
Acute coronary syndrome	4 (2.2)	2 (1.1)	0.388
Thalassemia	0 (0.0)	1 (0.5)	1.000
Corticosteroids use	1 (0.6)	0 (0.0)	0.492
Past COVID-19 infection	2 (1.1)	5 (2.7)	0.499
Concomitant medications, n (%)			
Acetaminophen	73 (40.8)	82 (44.3)	0.494
Naproxen	103 (57.5)	118 (63.8)	0.223
Famotidine	87 (48.6)	102 (55.1)	0.212
Diphenhydramine	61 (34.1)	75 (40.5)	0.203
Dextromethorphan	26 (14.5)	34 (18.4)	0.322
Bromhexine	39 (21.8)	43 (23.2)	0.740
Dimenhydrinate	8 (4.5)	10 (5.4)	0.680
Loperamide	13 (7.3)	14 (7.6)	0.912
Hydroxychloroquine	19 (10.6)	22 (11.9)	0.700
Multivitamins	52 (29.1)	58 (31.4)	0.633
Vit D 50000	43 (24.0)	49 (26.5)	0.589
Zinc	73 (40.8)	82 (44.3)	0.494
Chlordiazepoxide	14 (7.8)	18 (9.7)	0.520
Promethazine	4 (2.2)	6 (3.2)	0.556
Time from symptom onset to enrollment (day), median (IQR)	1.0 (1.0-2.0)	1.0 (1.0-3.0)	0.918
Time from symptom onset to diagnosis (day), median (IQR)	2.0(2.0-4.0)	3.0 (2.0-4.0)	0.096
Time from past Covid infection history and reinfection (day), median (IQR)	178.0 (178.0-178.0)	365.0 (300.0-365.0)	0.180

IQR: Interquartile range; SD: Standard deviation

Table 2 Comparison of effect of levamisole on primary and secondary outcomes in non-hospitalized patients with COVID-19

Clinical presentation	Control group (n=180)	Intervention group (n=185)	P
General health condition	**Median (IQR)**		
Day 1	6 (5-7)	6 (4-7)	0.365
Day 3	5 (4-6)	4 (3-5)	0.001
Day 5	4 (3-5)	3 (2-5)	0.000
Day 7	3 (2-4)	2 (1-4)	0.000
Day 9	2 (1-3)	1 (0-2)	0.000
Day 14	1 (0-2)	0 (0-1)	0.000
Fever, n (%)			
Day 1	84 (48.3)	91 (49.2)	0.863
Day 3	63 (36.2)	35 (19.2)	0.000
Day 5	35 (20.2)	19 (10.4)	0.010
Day 7	9 (5.2)	5 (2.7)	0.231
Day 9	2 (1.2)	2 (1.1)	1.000
Day 14	1 (0.6)	2 (1.1)	1.000
Chills, n (%)			
Day 1	47 (27.0)	64 (34.6)	0.120
Day 3	36 (20.8)	27 (14.8)	0.141
Day 5	17 (9.8)	10 (5.5)	0.124
Day 7	3 (1.7)	4 (2.2)	1.000
Day 9	2 (1.2)	2 (1.1)	1.000
Day 14	1 (0.6)	1 (0.5)	1.000
Cough, n (%)			
Day 1	71 (40.8)	100 (54.1)	0.012
Day 3	65 (37.4)	77 (42.3)	0.340
Day 5	52 (30.1)	57 (31.3)	0.797
Day 7	45 (26.0)	34 (18.7)	0.097
Day 9	28 (16.2)	19 (10.4)	0.110
Day 14	12 (6.9)	13 (7.1)	0.962
Fatigue, n (%)			
Day 1	86 (49.4)	98 (53.0)	0.502
Day 3	70 (40.2)	71 (39.0)	0.814
Day 5	52 (30.1)	44 (24.2)	0.212
Day 7	46 (26.6)	32 (17.6)	0.040
Day 9	33 (19.1)	22 (12.1)	0.069
Day 14	21 (12.1)	12 (6.5)	0.067
Headache, n (%)			
Day 1	57 (32.8)	74 (40.0)	0.154
Day 3	50 (28.7)	54 (29.7)	0.846
Day 5	36 (20.8)	27 (14.8)	0.141
Day 7	26 (15.0)	10 (5.5)	0.003
Day 9	16 (9.2)	5 (2.7)	0.009
Day 14	10 (5.8)	3 (1.6)	0.036
Myalgia, n (%)			
Day 1	83 (48.0)	92 (49.7)	0.740
Day 3	72 (41.4)	66 (36.3)	0.322
Day 5	55 (31.8)	45 (24.7)	0.139
Day 7	37 (21.4)	16 (8.8)	0.001
Day 9	18 (10.4)	7 (3.8)	0.016
Day 14	9 (5.2)	3 (1.6)	0.061
Sore throat, n (%)			
Day 1	51 (29.3)	57 (30.8)	0.757

Table 2 Comparison of effect of levamisole on primary and secondary outcomes in non-hospitalized patients with COVID-19 (continued)

Day 3	35 (20.1)	26 (14.3)	0.145
Day 5	19 (11.0)	18 (9.9)	0.736
Day 7	12 (6.9)	11 (6.0)	0.733
Day 9	8 (4.6)	8 (4.4)	0.917
Day 14	3 (1.7)	4 (2.2)	1.000
Rhinorrhea, n (%)			
Day 1	19 (10.9)	27 (14.6)	0.288
Day 3	11 (6.3)	11 (6.0)	0.913
Day 5	7 (4.0)	4 (2.2)	0.320
Day 7	3 (1.7)	1 (0.5)	0.361
Day 9	1 (0.6)	1 (0.5)	1.000
Day 14	1 (0.6)	2 (1.1)	1.000
Dyspnea, n (%)			
Day 1	19 (10.9)	12 (6.5)	0.135
Day 3	14 (8.0)	10 (5.5)	0.337
Day 5	2 (1.2)	6 (3.3)	0.174
Day 7	3 (1.7)	5 (2.7)	0.724
Day 9	1 (0.6)	3 (1.6)	0.623
Day 14	1 (0.6)	1 (0.5)	1.000
Anorexia, n (%)			
Day 1	30 (17.2)	33 (17.8)	0.882
Day 3	23 (13.2)	32 (17.6)	0.255
Day 5	21 (12.1)	13 (7.1)	0.110
Day 7	14 (8.1)	8 (4.4)	0.149
Day 9	7 (4.0)	4 (2.2)	0.315
Day 14	4 (2.3)	1 (0.5)	0.201
Nausea, n (%)			
Day 1	25 (14.4)	15 (8.1)	0.060
Day 3	22 (12.6)	15 (8.2)	0.174
Day 5	9 (5.2)	13 (7.1)	0.488
Day 7	4 (2.3)	6 (3.3)	0.751
Day 9	4 (2.3)	2 (1.1)	0.438
Day 14	0 (0.0)	1 (0.5)	1.000
Vomiting, n (%)			
Day 1	11 (6.3)	5 (2.7)	0.097
Day 3	4 (2.3)	0 (0.0)	0.056
Day 5	1 (0.6)	0 (0.0)	0.487
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	0 (0.0)	-
Day 14	0 (0.0)	0 (0.0)	-
Diarrhea, n (%)			
Day 1	16 (9.2)	17 (9.2)	0.998
Day 3	10 (5.7)	8 (4.4)	0.561
Day 5	3 (1.7)	1 (0.5)	0.361
Day 7	1 (0.6)	1 (0.5)	1.000
Day 9	1 (0.6)	0 (0.0)	0.487
Day 14	1 (0.6)	1 (0.5)	1.000
Hyposmia, n (%)			
Day 1	18 (10.3)	27 (14.6)	0.224
Day 3	17 (9.8)	26 (12.3)	0.191
Day 5	15 (8.7)	18 (9.9)	0.692
Day 7	16 (9.2)	12 (6.6)	0.354

Table 2 Comparison of effect of levamisole on primary and secondary outcomes in non-hospitalized patients with COVID-19 (continued)

Day 9	13 (7.6)	8 (4.4)	0.208
Day 14	8 (4.6)	4 (2.2)	0.199
Dysgeusia, n (%)			
Day 1	18 (10.3)	28 (15.1)	0.157
Day 3	18 (10.3)	28 (15.4)	0.156
Day 5	16 (9.2)	20 (11.0)	0.587
Day 7	15 (8.7)	10 (5.5)	0.242
Day 9	14 (8.1)	8 (4.4)	0.149
Day 14	5 (2.9)	6 (3.3)	0.839
Hospital admission, n (%)	4 (2.2)	2 (1.1)	0.444
Time between symptoms onset and hospitalization (days), median (IQR)	9.5 (8.0-9.5)	7.0 (7.0-7.0)	0.102

IQR: Interquartile range; SD: Standard deviation

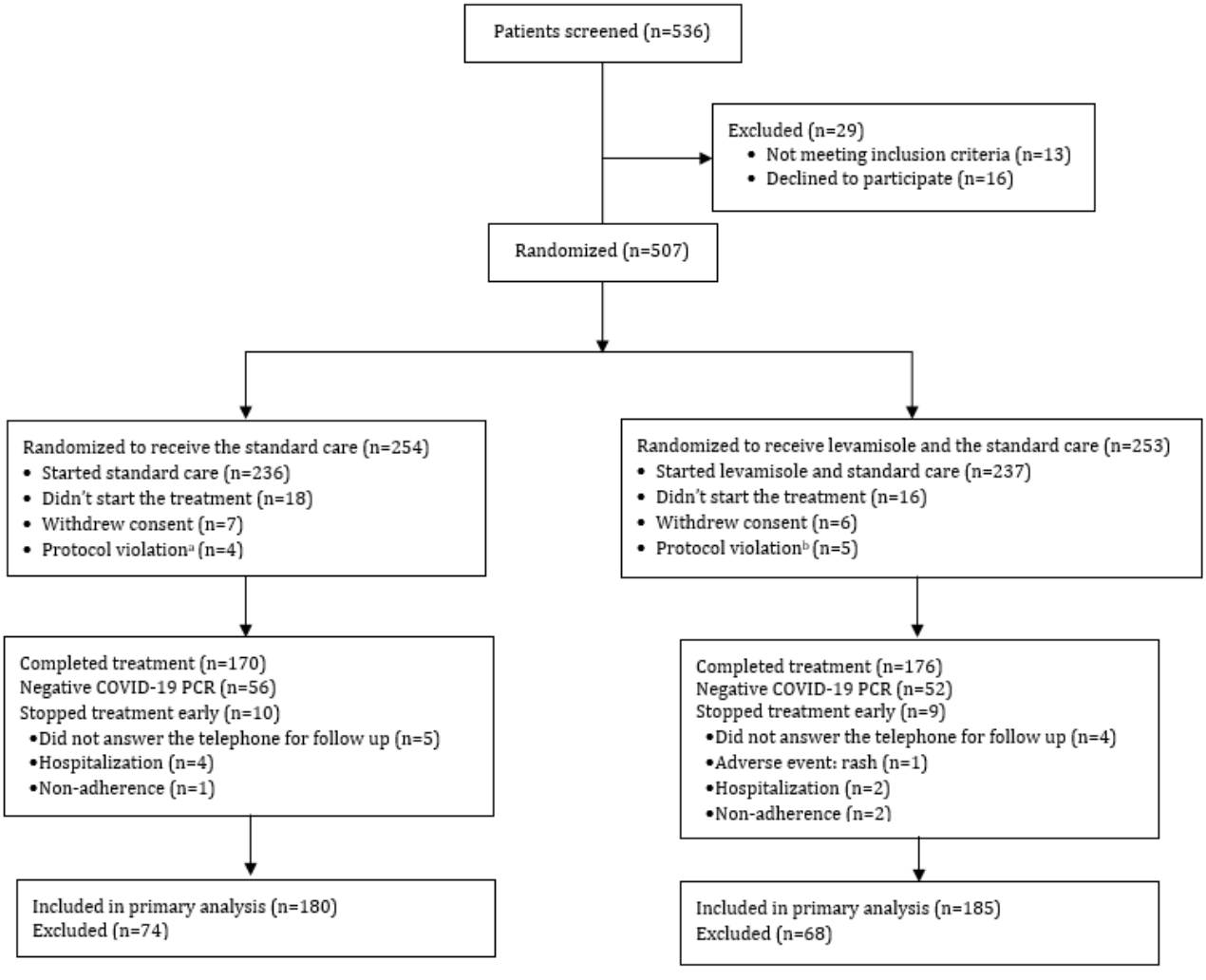

Figure 1 CONSORT flow diagram ([a] one patient had elevated liver enzymes, and one was pregnant; [b] three patients were lactating, one was pregnant, and one had low glomerular filtration rate)

Table 3 The odds ratio of clinical presentation of patients with COVID-19 comparing control and intervention groups by generalized estimating equations

Clinical presentation	OR	95% CI	P-value
Fever	0.260	0.113-0.599	0.002
Chills	0.223	0.076-0.648	0.006
Cough	0.638	0.376-1.083	0.096
Fatigue	0.576	0.346-0.960	0.034
Headache	0.613	0.371-1.013	0.056
Myalgia	0.544	0.317-0.932	0.027
Sore throat	0.651	0.248-1.712	0.384
Rhinorrhea	0.285	0.100-0.816	0.019
Dyspnea	0.495	0.095-2.573	0.403
Anorexia	0.771	0.372-1.594	0.482
Nausea	1.034	0.358-2.986	0.951
Vomiting	0.193	0.021-1.810	0.150
Diarrhea	0.966	0.263-3.552	0.959
Hyposmia	0.845	0.345-2.070	0.713
Dysgeusia	0.824	0.358-1.900	0.650

CI: Confidence interval; OR: Odds ratio

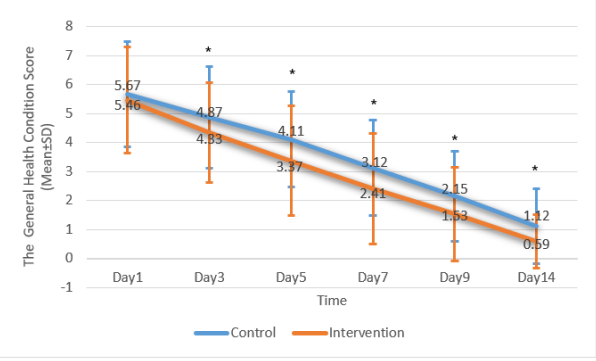

Figure 2 The general health condition of the patients measured by verbal numeric scale. The mean±SD subjective overall condition of the patients on 6 follow-up times is shown. Significance is indicated by * for P<0.001

tients in each group.

2.4. Randomization

In this study, patients were randomly assigned into two study arms by the permuted block randomization method. Six quadruple blocks, including AABB, ABAB, ABBA, BBAA, BABA, and BAAB were defined. Blocks were chosen randomly utilizing a random number table. According to the order specified in each block, two patients received treatment A (with LVM), and two patients received treatment B (without LVM). The appropriate number of vials of open-label study drugs were assigned to the patient. Sites did not have access to the randomization list and were unaware of the treatments sequence. At the health center, study medication was distributed according to the random number allocated to each participant. The research pharmacies held this list, and statisticians verified that the randomization sequence was followed.

2.5. Intervention

Patients were randomly assigned in a 1:1 ratio into intervention or control groups by the permuted block randomization method. The control group received only the standard COVID-19 care based on the ninth edition of COVID-19 diagnosis and treatment guideline at the level of outpatient and inpatient services in Iran, that was released on December 2019. Standard COVID-19 care included the hydroxychloroquine tablets 200 mg twice daily for five days, acetaminophen tablets 500 mg every 6 hours in case of fever, naproxen tablets 500 mg every 8 hours in case of myalgia, famotidine 40 mg daily, diphenhydramine syrup 10 cc every 8 hours in case of sore throat and cough, etc. (11). The intervention group received levamisole 50 mg/day for ten days in addition to the standard care.

2.6. Outcomes and follow-up

We used a predetermined checklist to collect both objective and subjective (self-reported) data, which included demographic characteristics such as age (year), gender (male/female), body mass index (BMI) (kg/m^2); the initial vital signs such as systolic and diastolic blood pressure (mmHg), temperature (°C), pulse rate (beats/min), respiratory rate (breaths/min), O2 saturation (percent), past medical history; and social history including cigarette smoking, alcohol drinking, opium or any drug abuse. The health care experts at the health centers were obliged to call the participants on days 1 (the medication start date), 3, 5, 7, 9,

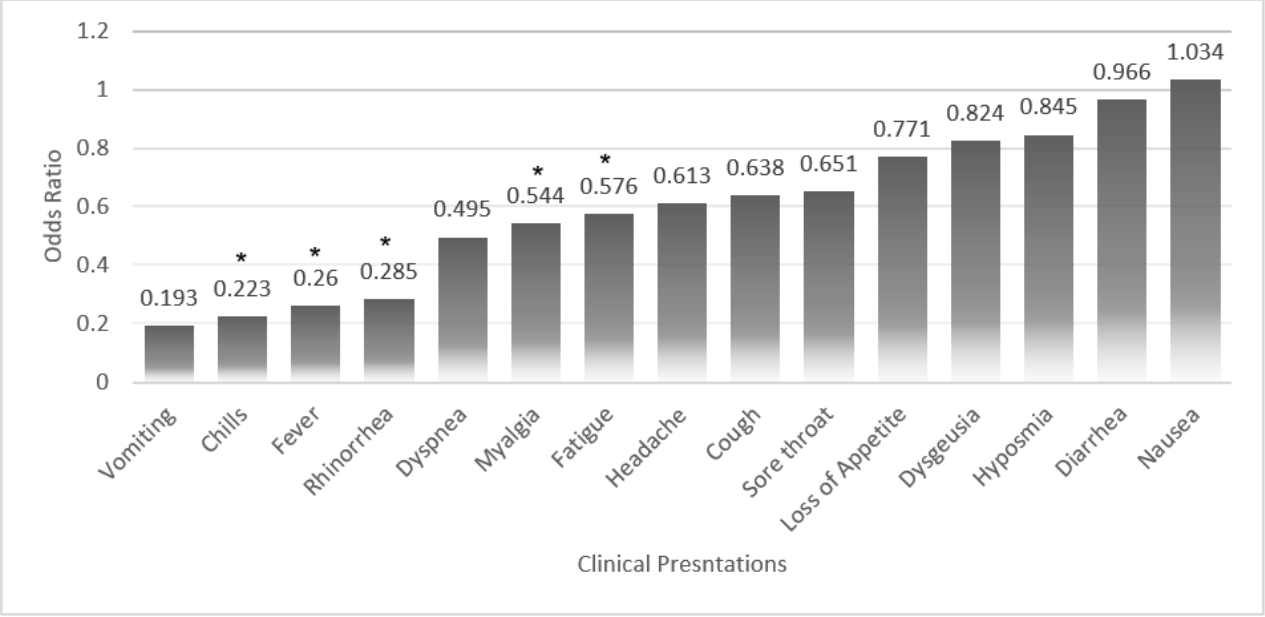

Figure 3 The odds ratio of COVID-19 related symptoms in the patients receiving levamisole and standard care comparing to the patients receiving only standard care. The odds ratios presented were measured by a generalized estimating equation. * Indicates P<0.05

and 14 to assess medication adherence, adverse events, presence and severity of COVID-19 symptoms, COVID-19 RT-PCR result, laboratory test results, hospitalization, and vital status. If participants were hospitalized within 14 days, we continued to monitor only their vital status; the clinical symptoms, adverse events, and lab results were not evaluated and not included in the statistical analysis. Due to the differences in treatment guidelines between hospitalized and non-hospitalized patients, the continuation of LVM prescription was not applicable after hospitalization. Moreover, our study aimed to determine the efficacy of LVM in non-hospitalized patients, its probable effect in the prevention of severe forms of the disease, and in general, its role in decreasing mortality and morbidity.

Clinical symptoms included the general condition of the patient, dyspnea, cough, diarrhea, nausea, vomiting, myalgia, fatigue, headache, sore throat, rhinorrhea, fever, anosmia, dysgeusia, anorexia, and loss of consciousness.

The general condition of the participants' score was self-assessed using an 11-point VNS (0 to 10, with 1-point increments) with 0 indicating no symptoms and 10 indicating death. Those who died of COVID-19 related complications were assigned a severity score of 10 for any surveys missed up until the date of death. During each follow-up, patients were educated on VNS and were informed of their previous given scores on each follow-up day.

We collected medication-related adverse events by direct questions on the most common adverse events. Investigators made a call or sent a text message to the participants or their designated third-party contacts who did not respond to follow-up surveys to ascertain outcomes. In case of unsuccessful attempts, investigators searched patients' vital statistics in the Integrated Health Record System. (so-called SIB). Agranulocytosis, anemia, and thrombocytopenia are uncommon adverse events of LVM. Therefore, we recommended physicians to obtain a complete blood count (CBC) for participants on days 1 and 14 to rule out such laboratory (lab) data abnormalities. However, since only 16 participants brought their lab data results, we didn't include them for analysis.

The health professionals were trained to immediately refer the patient to selected hospital centers in case of any of the following symptoms: 1) increase in respiratory rates, esp. > 24 breaths per minute, progressive course of shortness of breath, difficulty in breathing, chest pain, burning, or heaviness in the chest 2) peripheral cyanosis 3) changes in consciousness, drowsiness, and confusion. Participants were advised to consult a physician at the selected comprehensive health center if they experienced any of the following symptoms to determine whether they would require hospitalization or continue their treatment, or they need to add supportive therapies: 1) exacerbation of cough or occurrence of productive cough; 2) persistence or exacerbation of fever above 38.5 °C after five days; 3) severe diarrhea not responding to oral replacement therapy with water and electrolytes; and 4) severe anorexia.

2.7. Study endpoints

The initial primary outcome was the self-reported general health condition of the participants measured by VNS on days 3, 5, 7, 9, 14. Secondary outcomes were the presence of any clinical symptom at days 3, 5, 7, 9, 14, adverse events, hospitalization, and death.

Table 4 Comparison of adverse events between control and intervention groups

Adverse events	Control group (n=180)	Intervention group (n=185)	P
Stomachache, n (%)			
Day 1	0 (0.0)	5 (2.9)	0.336
Day 3	1 (1.8)	8 (4.7)	0.455
Day 5	1 (1.8)	4 (2.4)	1.000
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	0 (0.0)	-
Day 14	0 (0.0)	0 (0.0)	-
Vertigo, n (%)			
Day 1	0 (0.0)	1 (0.6)	1.000
Day 3	0 (0.0)	2 (1.2)	1.000
Day 5	0 (0.0)	1 (0.6)	1.000
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	1 (0.6)	1.000
Day 14	0 (0.0)	1 (0.6)	1.000
Skin rash, n (%)			
Day 1	0 (0.0)	0 (0.0)	-
Day 3	0 (0.0)	0 (0.0)	-
Day 5	0 (0.0)	1 (0.6)	1.000
Day 7	0 (0.0)	1 (0.6)	1.000
Day 9	0 (0.0)	1 (0.6)	1.000
Day 14	0 (0.0)	1 (0.6)	1.000
Metallic taste in mouth, n (%)			
Day 1	0 (0.0)	7 (4.1)	0.197
Day 3	0 (0.0)	7 (4.1)	0.196
Day 5	0 (0.0)	3 (1.8)	0.574
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	0 (0.0)	-
Day 14	0 (0.0)	0 (0.0)	-
Insomnia, n (%)			
Day 1	1 (1.8)	1 (0.6)	0.437
Day 3	0 (0.0)	0 (0.0)	-
Day 5	0 (0.0)	0 (0.0)	-
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	0 (0.0)	-
Day 14	0 (0.0)	0 (0.0)	-
Oral ulcer, n (%)			
Day 1	0 (0.0)	1 (0.6)	1.000
Day 3	0 (0.0)	1 (0.6)	1.000
Day 5	0 (0.0)	1 (0.6)	1.000
Day 7	0 (0.0)	0 (0.0)	-
Day 9	0 (0.0)	0 (0.0)	-
Day 14	0 (0.0)	0 (0.0)	-
Total patients with adverse events	2 (3.5)	21 (12.6)	0.052

2.8. Statistical analysis

Before analyzing the data, quantitative variables in terms of normality were examined through the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare the general health condition and age in the intervention group and control group. An independent sample t-test, tested the difference in BMI means between control and intervention groups. The presence of symptoms at each time point, gender, vital signs, social history, co-existing conditions, concomitant medications, time from symptom onset to enrollment and diagnosis, and the time from past COVID-19 infection history, reinfection, and adverse events were assessed

with the chi-square/Fisher exact test.

Generalized estimating equations (GEE), was used to test possible differences in the general health condition of patients (measured by VNS) and clinical presentations (using age groups and the baseline presentation as a covariate), assuming LVM and follow-up (when applicable) as fixed factors, with marginal distribution and considering the interaction between LVM prescription and follow-ups. Linear and binary logistic models were selected for the primary and secondary outcomes, respectively. The quasi-likelihood under independence model criterion (QIC) and corrected quasi-likelihood under independence model criterion (QICC) were used to choose between different correlation structures and consider various interactions. According to this criterion, the structure that obtained the smaller QIC or QICC was better. The odds ratio and 95% confidence interval (CI) for change in severity score from baseline between groups are presented. Mortality analysis was not performed because no death occurred during the study.

All the statistical analysis was conducted by SPSS software version 20 according to the intention-to-treat principle (that is, all participants with data are included in the analyses regardless of their medication status) with a 2-sided type I error using an α of 0.05.

3. Results

3.1. Patients' characteristics and baseline features

Figure1 shows the CONSORT flowchart of the study. Of 536 patients who consented and were assessed for eligibility, 507 patients underwent randomization and entered the study (237 patients started a 10-day course of LVM added to the standard care; 254 patients started a 10-day course of the standard care only). Of the 29 patients who were not randomized, 13 patients did not meet eligibility criteria, and 16 patients declined to participate. Thirty-four randomized patients did not receive treatment (13 patients withdrew consent, and 9 patients had protocol violations). One hundred eight randomized patients were excluded from the study during the follow-up due to their negative COVID-19 RT-PCR results.

Demographics and disease characteristics of the patients in the two groups showed no significant difference (Table 1). The mean age of the patients was 41.3±13.2 years, and 55.1% of the patients were male. Overall, 9.4% had diabetes, 8.8% had hypertension, 1.6% of patients had cardiovascular diseases, 1.1% had asthma, and 1.9% had past COVID-19 infection. At the screening phase, both groups had a median oxygen saturation of 96% while breathing room air (IQR: 95.0-97.0). The median time from symptom onset to study enrollment and diagnosis in both groups was 2.0 and 3.0 days, respectively (IQR: 1.0-3.0 and 2.0-4.0, respectively). Other than LVM, patients in the control and intervention groups were administered identical medications (Table 1).

3.2. Efficacy of levamisole treatment

LVM has significantly decreased the VNS and improved the general health condition of the patients compared to the control group (B=-0.635; CI: -0.041,-0.329; P<0.001) (Figure2). Moreover, as shown in table 2, when comparing each follow-up, on day 3, 5, 7, 9, and 14, patients randomized to the intervention group had significantly better general conditions than those randomized to the control group (P<0.001). The number of hospitalized patients in the control and intervention groups was 4 (2.2%) and 2 (1.1%), respectively (P=0.444), with a median time of 7.5 days between symptom onset and admission (IQR: 7.0-10.2 days). The mortality rate was not analyzed due to the zero number of events.

In the intervention group, patients had significantly lower odds of complaining from fever compared to the control group (OR=0.260; 95% CI: 0.113,0.599; P=0.002) (Table 3). When comparing febrile status on different days, we only found a significant difference in febrile status on days 3 and 5 (P< 0.05) (Table 2). Similarly, the odds of complaining from chills was significantly lower in patients receiving LVM with standard care, compared to the control group (OR=0.223; 95% CI: 0.076,0.648; P=0.006) (Table 3), but no significant difference was found when comparing two groups on each follow-up day (Table 2).

Baseline cough was significantly more reported in the intervention group than the control group (P=0.012) (Table 2); thereby, we considered adjusting cough at baseline in the GEE model. Following adjustment, we found that the odds of reporting cough was less in the intervention group than in the control group, but the difference was not significant (OR= 0.638; 95% CI: 0.376,1.083; P=0.096) (Table 3). In addition, when comparing two groups in cough on days 3, 5, 7, 9, 14, no significant difference was found (Table 2). The intervention group had significantly lower odds of fatigue (OR=0.576; 95% CI: 0.346,0.960; P=0.034) (Table 3). On day 7 of follow-up, the intervention group experienced less fatigue than the control group, which was statistically significant (P=0.044, table 2).

Patients in the intervention group had significantly lower odds of myalgia than the control group (OR=0.544; 95% CI: 0.317,0.932; P=0.027, table 3). 37 (21.4%) of patients in the control group complained of myalgia on day 7 while only 16 (8.8%) of the intervention group experienced myalgia (P=0.001); on day 9 this was reduced to 18 (10.4%) versus 7 (3.8%) in the control and intervention groups, respectively (P=0.016) (Table 2). Patients receiving LVM also had significantly fewer odds of rhinorrhea than those receiving only the standard care (OR=0.285; 95% CI: 0.100,0.816; P=0.019) (Table 3).

Patients in the intervention group reported fewer headaches over a follow-up on the 7th (P=0.003), 9th (P=0.009), and 14th (P=0.036) days than the control group (Table 2). The odds of experiencing headache was less in the intervention group; however, this finding was not statistically significant

(OR=0.613; 95% CI: 0.371,1.013; P=0.056) (Table 3).

We found no significant difference in dyspnea, diarrhea, nausea, vomiting, sore throat, hyposmia, dysgeusia, and anorexia at baseline and during the treatment (P>0.05, tables 2 and 3, and figure 3). Loss of consciousness was not reported in either group during the study period.

3.3. Adverse events

No severe adverse events were reported by patients in either group. Adverse events were experienced by only 23 (10.3%) of patients in the study, which were mild and self-limited, including stomachache (n=9), metallic taste in the mouth (n=7), vertigo (n=2), skin rash (n=1), and oral ulcer (n=1) (Table 4). In total, 2 (3.5%) of patients in the control group reported one or more adverse events while 21 (12.6%) of the intervention group experienced such events, but this difference was not significant (P=0.052) (Table 4).

4. Discussion

In this trial, individuals who underwent LVM therapy for ten days showed significantly improved clinical status on day 14 compared to those who received standard care in this clinical study of patients with mild to moderate COVID-19. The difference in the clinical status on days 3, 5, 7, 9, and 14 between the 10-day LVM and standard care groups was significant. We used an 11-point scale (VNS) to assess the participants' overall health as they were handled outpatient. Unlike hospitalized patients, our goal in cases of mild to moderate COVID-19 in the outpatient setting is to improve patient-reported health status. For instance, one may experience cough, but from their point of view, cough is tolerable. Although this is a subjective measurement, it is valuable. The odds of fever, chills, fatigue, myalgia, rhinorrhea were also found to be significantly lower in the intervention group. The difference in the occurrence of dyspnea, cough, diarrhea, nausea, vomiting, sore throat, hyposmia, dysgeusia, and loss of appetite was not significant.

The rate of hospitalization in our study was low (1.6%); this is in agreement with the previous trial on LVM (4). Our multicenter trial took place in primary health centers, so we assumed that patients with a better overall health condition visited our study sites. On the other hand, COVID-19 suspected patients with severe health conditions would directly go to the hospitals, bypassing the health centers. Moreover, we only included COVID-19 patients with mild to moderate illnesses in our trial, which could explain the low rate of hospitalization and mortality in our study.

The pro-inflammatory state is the second stage of COVID-19 illness, and it is associated with an increased level of inflammatory cytokines such as IL-6 and IL-8 leading to cytokine storm, systemic inflammation, and severe acute respiratory syndrome (13).

Therefore, researchers have focused on reducing inflammatory response as a potential therapeutic target against the second phase of COVID-19 disease. Many current studies are attempted to identify intracellular and molecular mechanisms and intervene to prevent COVID-19 illness from progressing to the second phase. In this regard, several studies evaluated the effect of LVM by explaining the molecular mechanisms. One study confirmed that LVM has a potential inhibitory effect on the papain-like protease of the shell of the virus (which is necessary for virulence of COVID-19), can decrease the levels of tumor necrosis factor alpha (TNF α) and interlukin-6 (IL-6), and as a chemical adjutant, can introduce the virus to the immune system and might help manage COVID-19 (7). Moreover, it was reported that LVM has an immune-enhancing effect, thereby increasing host immune response and viral clearance. Furthermore, Al-Kuraishy et al. declared that co-administration of LVM with the COVID-19 vaccine might enhance the humoral immune response and immunization against SARS-CoV-2 (6).

So far, few specific antiviral and immunomodulatory treatments are available for COVID-19. Anti-inflammatory and immunomodulatory treatments have been proven in studies to help manage COVID-19 patients who are in the pro-inflammatory stage of the disease (14). In severe cases of COVID-19, IL-6 levels in the blood are notably high (15). Using tocilizumab (TCZ), a human IL-6 blocking medication, reduced C-reactive protein (CRP), oxygen demand, the opacity of the lung lesion, and normalized the number of lymphocytes in 84.2, 75, 90.5, and 52.6 percent of COVID-19 patients, respectively (16). Another anti-IL-6 agent is LVM. This medication inhibits IL-6's pro-inflammatory action, and COVID-19 patients may benefit from this treatment.

With the same thought, several clinical trials on LVM in COVID-19 patients have been registered (17-20), but the results are not yet published. Unlike the previous study (4), which found a significantly better cough status on days 3 and 14 in patients randomized to the LVM group, we found no significant difference in reducing cough between the two groups. We have to mention that our statistical analysis method was far more than only running a chi-square in SPSS to remove the effect of time and other probable interactions. Therefore, apart from chi-square/Fisher's exact test, which showed no difference in each follow-up comparison, we used a GEE model in the current study and confirmed no difference in the odds of developing cough between the two groups.

The previous trial (4) reported no significant differences in febrile status on days 1, 3, 7, and 14 between the intervention and control groups which contradicts our findings. We found significantly lower odds of reporting fever and chills in the intervention group. The current study showed that the odds of not being feverish or not developing chills were almost 0.7. Moreover, fever was less reported on days 3 and 5 of follow-up in the intervention group. Several factors may account for the lack of difference in the aforementioned outcomes observed in the 3-day LVM added to the standard care group in the previous study. Given the small sample size of the previous research and the low dose of LVM continued for

only three days, the actual antifebrile effect of LVM was not seen in the previous study.

Myalgia was also assessed in the previous study (4); but unlike the current study, they found no significant difference. Interestingly, only five (6%) patients in the previous study (4) were complaining of myalgia at the initial phase, which is significantly fewer than the present study with 175 (48.9%) patients with myalgia. The discrepancy in findings between the two trials might be explained by the prior study's limited sample size and the inability to detect an efficacy outcome difference. Also, it might have happened since the previous study was conducted in 2020 with different variants of COVID-19 and less tendency of the virus to cause myalgia.

The previous trial (4) reported significant differences between two groups in dyspnea after 7th and 14th days of follow-up, whereas the current study found no difference comparing days 3, 5, 7, 9 and 14 of follow-ups. Although the odds of dyspnea were lower in the present study's intervention group, this finding was not statistically significant. Comparing the baseline dyspnea between the two studies, the percentage of patients with dyspnea in the previous study (n=27, 54.0%) is higher than in the current study (n=31, 8.6%). This might have happened due to the methodology and difference in inclusion criteria; so that patients with a poorer health condition were included in the previous study in contrast to the current study, that wide range of patients with O2sat>92% including both mild and moderate conditions with or without dyspnea were included. Only a few adverse events were reported in our trial, which is congruent with the previous study. However, further studies with a larger sample size need to confirm our findings.

5. Limitations

This trial has several limitations. First, COVID-19 RT-PCR diagnostic testing was limited, non-hospitalized patients were often ineligible for testing, and the turnaround time for results was multiple days. Second, the relatively low sample size in this trial made it impossible to calculate hospital length of stay, ICU admission, and mortality. Future trials should consider studying mortality and hospitalization rate in a higher study population. Third, since the patients had several coexisting diseases and were subjected to a various medication regimen, the results could have been affected by the heterogeneity of the sample and its treatment. Fourth, the patients were given a dose of only 50 mg LVM after symptom onset to minimize the risk of adverse events and better tolerance. The last limitation is that laboratory variables that could be used in identifying additional predictors of patients' outcomes were not collected. More objective methods such as comprehensive laboratory evaluation that contribute to distinguishing patients progressing to severe and critical COVID-19 in both groups remain to be elucidated.

6. Conclusion

Among patients with mild to moderate COVID-19, those randomized to a 10-day course of LVM added to the standard care had a statistically significant difference in general health status compared with standard care at 14 days after initiation of treatment. Patients randomized to a 10-day course of LVM added to the standard care had statistically significant odds of not developing a fever, chills, fatigue, myalgia, and rhinorrhea compared with standard care at 14 days after initiation of treatment.

7. Declarations

7.1. Acknowledgment

We thank all the medical team at the health centers, including doctors, nurses, the health care experts, and the staff who cooperated with this trial. We thank Dr. Jafar Sadegh Tabrizi, Dr. Hengameh Namdaritabar, Dr. Mojgan Anvari, Dr. Ali Nikfarjam, Dr. Jamshid Motamed, Dr. Zahra Neda, and Dr. Najmeh Amini in the Ministry of Health and Education for facilitating the implementation of the study in the health centers. We thank Dr. Nahid Zomorrodin, Dr. Maryam Vali, Dr. Fatemeh Nasri, Dr. Mahboobe Ghanbarian, Dr. Mehdi Jalali, Dr. Mehran Eskandari, Farzaneh Ashouri, and Forough Shahrabi Farahani for visiting the patients and completing their follow-ups. We also thank the patients and their families for their cooperation. Preprint of this clinical trial had been uploaded in Research Square previously. (DOI: https://doi.org/10.21203/rs.3.rs-964097/v1)

7.2. Authors' contributions

All authors contributed to the study's conception and design. Material preparation and data collection were performed by MHA, AZD, BK and AB. The design of the work was performed by HEK, AB, HK, MHA and MA. MHKG performed the analysis. MHA developed the initial draft of the manuscript, and other authors provided feedback on prior drafts. All authors read and approved the final manuscript.

7.3. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

7.4. Funding

AJA University of Medical Sciences supported the manuscript with the grant number (IR.AJA.REC.1399.199), Tehran, Iran.

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