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ORIGINAL ARTICLE

Tranilast as an Adjunctive Therapy in Hospitalized Patients with Severe COVID-19: A Randomized Controlled Trial

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Background. Tranilast is a potential NLRP3 inflammasome inhibitor that may relieve progressive inflammation due to COVID-19.

Aim of the study. To evaluate the therapeutic effects of Tranilast in combination with antiviral drugs in non-ICU-admitted hospitalized patients with COVID-19.

Methods. This study was an open-label clinical trial that included 72 hospitalized patients with severe COVID-19 at Razi Hospital, Ahvaz, Iran, from July 2020–August 2020. These patients were randomly assigned in a 1:1 ratio to control (30) and intervention groups (30). Patients in the control group received antiviral therapy, while patients in the intervention group received Tranilast (300 mg daily) in addition to the antiviral drugs for seven days. The collected data, including the expression of inflammatory cytokine, laboratory tests, and clinical findings, was used for intragroup comparisons.

Results. The intervention group showed significantly lower levels of NLR ($p = 0.001$), q-CRP ($p = 0.002$), IL-1 ($p = 0.001$), TNF ($p = 0.001$), and LDH ($p = 0.046$) in comparison with the control group. The effect of intervention was significant in increasing the O_2 saturation ($F = 7.72$, $p = 0.007$). Long hospitalization (four days or above) was 36.6% in the Tranilast and 66.6% in the control group (RR = 0.58; 95% CI: 0.38–1.06, $p = 0.045$). In the Tranilast and control groups, one and four deaths or hospitalization in ICU were observed respectively (RR = 0.31; 95% CI: 0.03–2.88, $p = 0.20$).

Conclusions. Tranilast might be used as an effective and safe adjuvant therapy and enhance the antiviral therapy's efficacy for managing patients with COVID-19. © 2022 Instituto Mexicano del Seguro Social (IMSS). Published by Elsevier Inc. All rights reserved.

Key Words: COVID-19, Tranilast, NLRP3 inflammasome, SARS-CoV-2, Cytokine storm.

Introduction

Coronavirus disease 2019 (COVID-19) was first reported in December 2019 in Wuhan, China (1). Up to January 16, 2022, more than 300 million confirmed cases of COVID-19, as well as more than 5.5 million cases of related death, have been reported globally. Most cases of COVID-19 de-

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velop a mild disease and recover without serious complications or hospitalization. However, 10–15% of the patients develop the severe form of the disease, leading to pulmonary involvement, acute respiratory distress syndrome (ARDS), Acute Lung Injury (ALI), or multi-organ failure (2). Since the beginning of the pandemic, several clinical trials have investigated the effectiveness of various medications and therapeutic strategies in this disease, with clinical trials still being conducted (3). Given the present critical situation and the importance of saving time and costs, some strategies, such as drug repurposing, have been considered (4). Recently U.S. Food and Drug Administration issued an emergency use authorization (EUA) for Merck's molnupiravir and Pfizer's Paxlovid for the treatment of mild-to-moderate coronavirus disease (COVID-19). However, no definite treatment has been introduced for COVID-19 yet. Therefore, there is an urgent need for safe and effective medications, as well as extensive vaccination coverage, regarding the high transmissibility of the virus, especially the novel and mutated variants. COVID-19 has severe impacts on the innate and adaptive immune responses. Interestingly, this virus can interfere with the antiviral responses, particularly IFN-1, leading to an inflammation exacerbation and subsequent cytokine storm, the main cause of death for most of the patients (5,6).

As a key component of innate immunity, overstimulation of the NLRP3 inflammasome can lead to a type of inflammatory cell death, known as pyroptosis, and overproduction of different cytokines, such as IL-1 and IL-18. According to studies, the NLRP3 inflammasome plays a critical role in the pathogenesis of β -coronaviruses, such as SARS-CoV, MERS-CoV, and SARS-CoV-2, increasing their virulence. Thus, it can be used as a potential therapeutic target in COVID-19, particularly in combination with antivirals (6,7). Recently published articles conducted on various aspects of the role of Inflammasomes in covid19 and the potential role of the NLRP3 Inflammasomes (8–10). A recent study has interestingly shown Specific suppression of the NLRP3 inflammasome repress immune overactivation and diminish COVID-19 like pathology in mice (11). It is very likely that suppressing the factors involved in the progressive inflammation, interfering with the innate immune responses, and inhibiting the viral replication together can be used as an effective therapeutic strategy in COVID-19. Tranilast (TR, N-[30,40-dimethoxycinnamoyl]-anthranilic acid) is a tryptophan metabolite analog and is reported to have inhibiting effects in homologous passive cutaneous anaphylaxis. Moreover, it has been used in treating various inflammation-mediated diseases, such as bronchial asthma, atopic dermatitis, allergic conjunctivitis, and hypertrophic scars (12). In addition to other NLRP3 inhibitors and immunomodulators, Tranilast is considered as a potential treatment for managing progressive inflammation in patients with severe COVID-19. Our recently published

study, the cornerstone of the present study, used the findings of several studies to provide a conclusion on the inflammation-inhibiting role of Tranilast, particularly on the NLRP3 inflammasome and other key factors involved in the immunopathogenesis of COVID-19 (13). However, the clinical and laboratory data on the application of Tranilast in the COVID-19 treatment are very limited. Thus, the present Randomized Controlled Trial (RCT) is intended to evaluate the therapeutic effects of Tranilast in combination with antiviral drugs in non-ICU-admitted hospitalized patients with COVID-19.

Study Design and Participants

This study was an open-label, single-center, randomized controlled trial assessing the safety and efficacy of Tranilast in adult COVID-19 patients admitted to the Razi Hospital, Ahvaz, Iran, from July 2020–August 2020. First, 72 patients were chosen, 12 of whom were excluded due to lack of meeting the eligibility criteria or their unwillingness to participate in the study. Hence, the data of 60 patients were included for the analysis. The inclusion criteria of the present study included hospital admission during the last 48 h, age of 18 years or older, receiving continuous O₂ therapy using reservoir bags, arterial blood O₂ saturation below 93%, lung involvement with crazy paving pattern, ground glass opacities, unilateral or bilateral consolidation, and a confirmed COVID-19 diagnosis by nasopharyngeal swab RT-PCR. Exclusion criteria of the present study included pregnant or breastfeeding women, patients with underlying neurological diseases, renal failure, or chronic hepatitis, patients taking anti-inflammatory, antioxidant, or immunosuppressant medications, patients with a history of allergy to Tranilast, and patients who have already participated in another clinical trial during the last month. The Ethics Committee approved the study of the Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1399.050). Furthermore, this study was registered in the Iranian Registry of Clinical Trials (IRCT20200419047128N1). The study protocol and objectives were explained to patients or/and their legal representatives, and written informed consent was taken before the interventions.

Randomization and Procedures

The patients were randomly assigned to control and intervention groups ($n = 30$ in each group) utilizing a random permuted block design using the combined analysis. Patients in the control group received favipiravir and/or remdesivir antiviral therapy based on Iran's national protocols for managing patients with COVID-19, while patients in the intervention group received Tranilast (300 mg TDS) besides the routine protocols. Supportive care was provided for patients in both groups, including fluid therapy, deep

Table 1. Dosage and time of antiviral drugs, Tranilast and complementary therapies used in the study.

	Intervention group	Control group	Dosage and time
Tranilast (Takeda Pharmaceutical Co. Japan)	✓	-	300 mg daily for 7 d (100 mg PO TDS ^a)
Antiviral drugs			
Remdesivir (Ronak Pharmaceutical Co. Tehran, Iran)	✓	✓	First day: 200 mg (IV injection) Second to fifth day: 1200 mg (600 mg PO BD ^b)
Favipiravir (Cytovex, Abidi Pharmaceutical Co. Tehran, Iran)	✓	✓	First day: 3200 mg (1600 mg PO BD) Second to fifth day: 1200 mg (600 mg PO BD)
Supportive treatments			
Vitamin. D	✓	✓	1000 IU PO daily
MgSO ₄	✓	✓	250 mg PO BD (based on serum Mg level)
Famotidine	✓	✓	40 mg PO daily
Zinc sulfate	✓	✓	30 mg PO daily
Vitamin C anticoagulants	✓	✓	500–1000 mg PO daily
Heparin glucocorticoids	✓	✓	Heparin 5000 IU, SC, TDS
Dexamethasone	✓	✓	8 mg daily IV injection

IV, Intravenous; Co, Company; mg, Milligrams.

^aTDS (or TD or TID), Three times a day.

^b“PO” means the medication is taken by mouth “bid” or twice a day.

vein thrombosis prophylaxis, stress ulcer prophylaxis, antibiotic therapy, and electrolyte disturbance management according to the hospital protocols. All patients were investigated and treated for seven days. Patients who finished the study were followed for an additional 14 days. The supportive therapies, medication dosage, and the study stages are shown in (Table 1). Patients were investigated daily using a checklist by trained nurses, and their complications and clinical condition were recorded. Peripheral blood sampling was done in both groups before and after the intervention. Lastly, the laboratory and clinical findings before, during, and after the intervention were used for data analysis. The cytokines serum levels, including TNF α , IL-1 β , and IL-6, were measured by the ELISA kits from Karmania Pars Gene Company, Kerman, Iran (Cat numbers: KPG-hTNF- α , KPG-hIL-1 β , and KPGhIL-6).

Outcomes

Primary outcomes of the present included ICU admission, mortality, discharge, duration between day 7 of the study and discharge, and laboratory measures, including ESR and CRP. Secondary outcomes of the present study were the discharge day, Neutrophil-to-Lymphocyte Ratio (NLR), blood oxygen saturation, IL-1, IL-6, and TNF- α levels, in addition to the safety-related outcomes, such as the adverse effects, laboratory abnormalities, serious infections, and Tranilast premature discontinuation.

Statistical Analysis

We performed a pilot study assessing the levels of d-dimer, IL-1, TNF- α , and CRP in 10 patients with COVID-19 who were divided into the intervention and control groups. Then, the mean and SD of these parameters were put into

the sample size calculation formula in two independent groups using the G*Power 3.1 software. The effect size (d) was estimated, the significance level was considered at 5%, and the statistical power was set at 80%. Finally, a maximum of 25 patients for each group was selected, with a population proportion (K) of 1. A 15% dropout was considered due to the possibility of lack of patient adherence. Thus, the sample size was increased to 30 per group.

$$n \geq \frac{(1+k)}{k} \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{d^2} + \frac{z_{1-\alpha/2}^2}{2(1+k)};$$

$$k = 1, \alpha = 0.05, d = 0.79 \Rightarrow n \geq 25$$

Continuous variables were reported in the median and Interquartile Range (IQR), while the categorical ones were described using the absolute frequency and percentage (%). For data analysis, the normal distribution of the quantitative variables was first assessed using the Kolmogorov-Smirnov test. The data did not have a normal distribution ($p < 0.05$). Thus, the Mann-Whitney U test was used for quantitative data comparisons, while the χ^2 and Fisher's exact tests were used for qualitative data comparisons. All the analyses were conducted using the SPSS version 19 with a significance level of 5%.

One-way ANOVA/ANCOVA was used to compare the O₂ saturation level between intervention and comparison groups. In this statistical model, we assessed the O₂ saturation measure on the seventh day as a dependent or outcome variable, O₂ saturation measure on the first day (baseline level) as the covariance, and intervention and comparison groups as the factor (independent variable). The mean difference (MD) and standardized mean difference (SMD) were considered as the effect size indicators (14).

Among the primary outcomes, the composite outcome, i.e., the occurrence of ICU admission or mortality outcomes, was estimated using crude statistical analysis, and

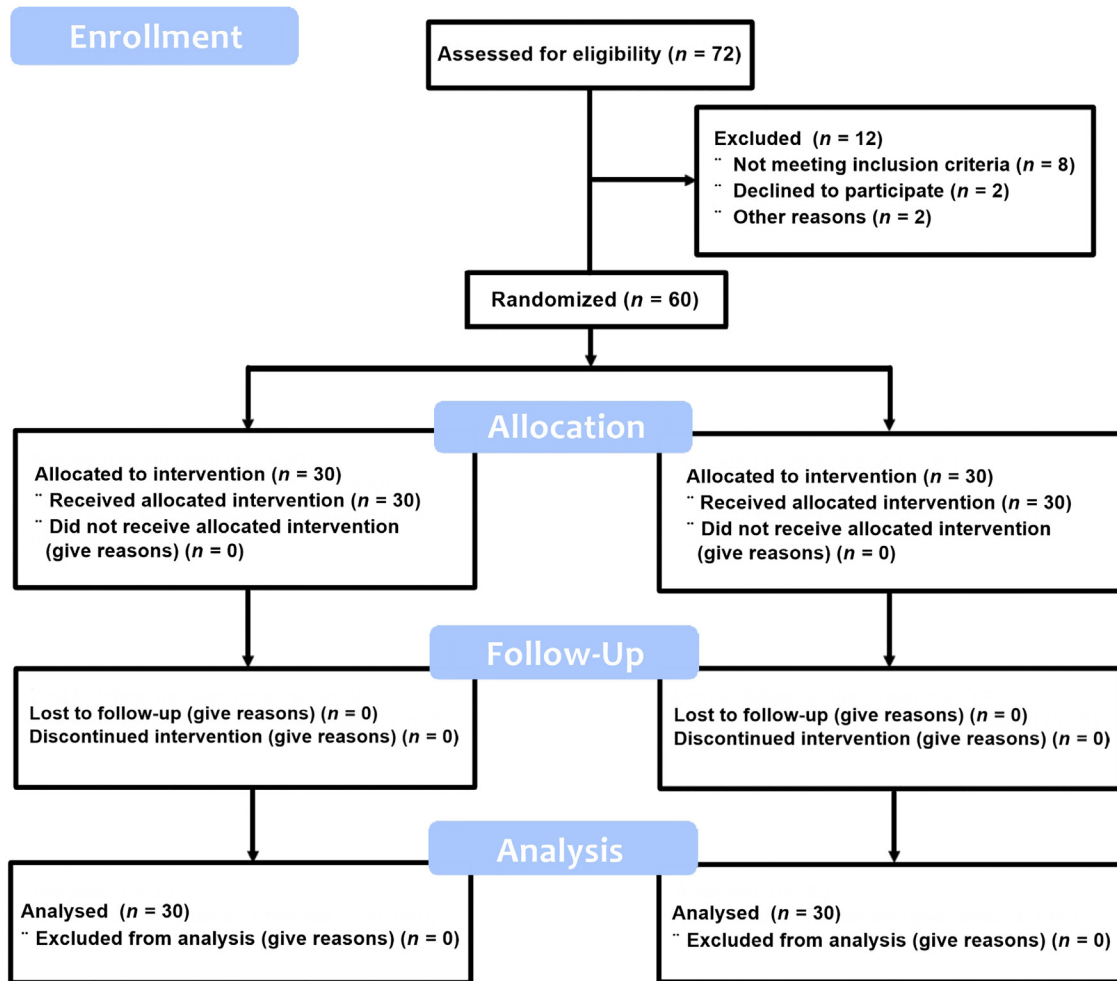


Figure 1. CONSORT flow diagram of patients enrolled through the clinical trial.

adjusted statistical analysis was conducted to consider each underlying disease, diabetes, cardiovascular disease, and kidney and liver diseases. Statistical analysis of binary outcomes was performed using log-binomial regression (15). To implement this statistical analysis, we used the “binreg” module in the STATA 14.2 software (Stata Corp. 2015. Stata Statistical Software: Release 14. College Station, TX: Stata Corp LP). The Risk ratio in the log-binomial regression model was interpreted based on Olivier et al (16).

Results

Seventy-two COVID-19 patients were assessed for eligibility, 12 of whom were excluded due to lack of meeting study inclusion criteria or their unwillingness for study participation. The 60 remaining patients with severe COVID-19 were randomly assigned to two groups (Figure 1). Considering the potential dropout rate of 15%, 60 patients ($n = 30$ in each group) completed the study whose data was included for data analysis. Although the duration of

intervention was seven days, the patients were followed up for an additional 14 d. Complete Blood Count and coagulation and biochemical assessments, including SGOT, SGPT, BUN, Cr, total and direct Bilirubin, ESR, quantitative CRP, LDH, and D-dimer, were assessed in both groups before and after the intervention. Furthermore, their blood Oxygen saturation was monitored and recorded every day.

The age (median \pm IQR) of patients in the control and intervention group were 59.5 ± 13.75 and 59.5 ± 15.25 years, respectively. Furthermore, 38 patients (62.3%) were males. The patients’ most common comorbidities included diabetes mellitus, cardiovascular diseases, and hypertension. The duration (median \pm IQR) between the symptomatic onset of the disease and randomization were 7 ± 2 and 6.5 ± 1.25 days in the control and intervention groups, respectively. Tables 2 demonstrate the demographic and pre-intervention clinical findings of the patients in both groups. Furthermore, no significant intergroup differences were found in terms of the values of all variables before the intervention ($p > 0.05$), including both qualitative and

Table 2. Demographics and clinical characteristics and Laboratory Finding of COVID-19 patients at baseline.

	Intervention group (n = 30)	<i>p</i> ^a	Control group (n = 30)
Age, median (IQR) years	59.5 (15.25)	59.5 (13.75)	0.838
BMI, median (IQR) kg/m ²	26.7 (4)	25.2 (4)	0.065
Time from symptom onset to randomization, median (IQR), days	6.5 (1.25)	7 (2)	0.366
Sex	Patients, No. (%)		
Male	20 (66.7)	18 (60)	0.39
Female	10 (33.3)	12 (40)	
Comorbidities	Patients, No. (%)		
Hypertension	8 (26.7)	0.11	14 (46.7)
Diabetes	5 (16.7)	11 (36.7)	0.14
Cardiovascular diseases	4 (13.3)	6 (20)	0.73
Kidney disease	1 (3.3)	3 (10)	0.61
Liver disease	0 (0)	1 (3.3)	1
Signs and symptoms	Patients, No. (%)		
Cough	28 (93.3)	27 (90)	0.40
Chest pain	24 (80)	25 (83.3)	0.739
Headache	21 (70)	21 (70)	1
Weakness and fatigue	21 (70)	22 (73.3)	0.77
Muscular pain	24 (80)	21 (70)	0.37
Chilling	0.426	20 (66.7)	17 (56.7)
Sore throat	0.41	22 (73.3)	19 (63.3)
GI symptoms	5 (16.7)	7 (23.3)	1
hospital admission, requiring high-flow nasal cannula or non-invasive mechanical ventilation	0 (0)	0 (0)	1
hospital admission, requiring supplemental oxygen	30 (100)	30 (100)	1
Laboratory findings	Median. (IQR)		
WBC (10 ³ /μL)	7.35 (5.25)	7.23 (2.78)	0.965
NLR	3.37 (1.15)	3.5 (1.19)	0.674
RBC	4.4 (1)	4.39 (0.6)	0.49
HCT (%)	36.55 (6)	36.88 (4.63)	0.30
HBG (g/dL)	12.9 (2.25)	12.2 (2)	0.162
PLT (10 ³ /μL)	220.5 (76)	118.5 (77)	0.51
q-CRP (mg/L)	43.5 (43)	40.5 (41)	0.739
D-dimer (ng/mL)	605 (442)	517 (361)	0.299
ESR (mm/h)	30 (6.25)	30 (5.5)	0.733
SGPT (U/L)	40 (26.25)	38.5 (22.25)	0.56
SGOT (U/L)	42 (41)	35 (22)	0.201
Direct Bilirubin (mg/dL)	1 (0.13)	0.9 (2)	0.062
BUN (mg/dL)	16 (11.5)	16 (8.5)	0.772
Cr (mg/dL)	1.05 (0.5)	1 (0.32)	0.601
LDH (U/L)	625 (230)	518.5 (360)	0.564
IL-1β (pg/mL)	10.16 (7.5)	8.02 (5.77)	0.131
IL-6 (pg/mL)	12 (8.64)	11.37 (7.77)	0.464
TNF-α (pg/mL)	22.32 (5)	21.32 (4)	0.214

COVID-19, coronavirus disease 2019

^a*p* values indicate differences between patients in the intervention group and the control group. *p* < 0.05 was considered statistically significant. The data did not have a normal distribution (*p* < 0.05). Thus, the Mann-Whitney *U* test was used for quantitative data comparisons, while the χ^2 and Fisher's exact tests were used for qualitative data comparisons. COVID-19, coronavirus disease 2019; WBC, White blood cells; RBC, red blood cells; HCT, hematocrit; Hb, Hemoglobin; NLR, Neutrophil-lymphocyte ratio; q-CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; SGPT, (Serum) glutamic pyruvic transaminase; SGOT, (serum) glutamic oxaloacetic transaminase; BUN, Blood urea nitrogen; IL-1β, Interleukin 1 beta; TNF-α, tumor necrosis factor alpha; IL-6, Interleukin 6; Cr, creatinine; No., number; IQR, interquartile range.

quantitative variables. All of patients required respiratory support at the time of randomization. None of the patients in both groups were intubated at baseline (Table 2). one patient (3.33%) and 3 (10%) who were admitted to the ICU and belonged to the Tranilast, and control groups respectively underwent non-invasive mechanical positive pressure ventilation. Non-invasive mechanical positive pressure ven-

tilation using a ventilator was conducted for 2 (6.66%) patients in the control group.

Table 3 presents the post-intervention patient outcomes and clinical findings of both groups. This study showed the significant improvement of patients in the intervention group in terms of weakness and fatigue compared to the control group (40% vs. 66.66%, *p* = 0.038), while no

Table 3. Clinical characteristics and outcomes of COVID-19 patients at post-intervention.

Characteristics	Intervention group (n = 30)	Control group (n = 30)	<i>p</i> ^a
Signs and symptoms	Patients, No. (%)		
Cough	5 (16.66)	10 (33.33)	0.131
Chest pain	2 (6.66)	4 (13.33)	0.389
Headache	4 (13.33)	5 (16.66)	0.718
Weakness and fatigue	12 (40)	20 (66.66)	0.038
GI symptoms	0 (0)	0 (0)	1
Myalgia	2 (10)	4 (13.33)	0.68
Rhinorrhea	0 (0)	0 (0)	1
Sore throat	1 (3.33)	5 (16.66)	0.85
Chilling	0 (0)	0 (0)	1
Laboratory findings	median (IQR)		
WBC (10 ³ /μL)	7.65 (3.25)	8 (3.85)	0.605
NLR	1.615 (0.52)	2.795 (1.05)	0.001
PLT (10 ³ /μL)	267 (150)	216.5 (135)	0.085
PT (S)	13 (0.5)	13 (1)	0.476
PTT (S)	32 (4.5)	32 (3.5)	0.720
q-CRP (mg/L)	21.6 (21)	38 (35)	0.002
ESR (mm/h)	11 (3)	12 (3)	0.014
D-dimer (ng/mL)	300 (160)	450 (313)	0.034
LDH (U/L)	395 (210)	500 (395)	0.046
SGPT (U/L)	41 (33.5)	33.5 (23.5)	0.097
SGOT (U/L)	32.5 (39.25)	33.5 (21)	0.589
BUN (mg/dL)	17 (10)	18 (9.25)	0.982
Cr (mg/dL)	1.05 (0.52)	1.05 (0.42)	0.988
Direct Bilirubin (mg/dL)	1 (0.2)	0.9 (0.2)	0.091
Pro-inflammatory cytokines	median (IQR)		
IL-1β (pg/mL)	6.1 (5.71)	10.21 (6.75)	0.001
TNF-α (pg/mL)	16.1 (4)	19.5 (5)	0.001
IL-6 (pg/mL)	10 (3.8)	9.11 (6.5)	0.145

COVID-19, coronavirus disease 2019; NLR, Neutrophil-lymphocyte ratio; PT, Prothrombin Time; PTT, partial thromboplastin time; q-CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; SGPT, (Serum) glutamic pyruvic transaminase; SGOT, (serum) glutamic oxaloacetic transaminase; BUN, Blood urea nitrogen; Cr, creatinine; IL-1β, Interleukin 1 beta; TNF-α, tumor necrosis factor alpha; IL-6, Interleukin 6; NIPPV, non-invasive positive pressure ventilation; No., number; IQR, interquartile range

^a*p* values indicate differences between patients in the intervention group and the control group. *p* < 0.05 was considered statistically significant.

significant intergroup difference was observed in terms of other symptoms. Regarding the laboratory findings, the intervention group showed significantly lower levels of ESR (*p* = 0.014), NLR (*p* = 0.001), d-dimer (*p* = 0.034), q-CRP (*p* = 0.002), IL-1 (*p* = 0.001), TNF (*p* = 0.001), and LDH (*p* = 0.046) in comparison with the control group. Nevertheless, as shown in Table 3, no significant difference was observed between the two groups regarding SGOT (*p* = 0.589), SGPT (*p* = 0.097), Cr (*p* = 0.988), and BUN (*p* = 0.982). Also, no adverse effect was reported in the patients in the intervention group during the study.

Figure 2 depicts the data regarding oxygen saturation during the study in both groups. In the analysis of O₂ saturation, the role of intervention in changing the mean of this quantitative outcome was statistically significant in the variance-covariance model (*F* = 7.72, *p* = 0.007). However, MD (the mean difference of this outcome between intervention and comparison groups) was 0.98 (95% CI: 0.27–1.68). Although this analysis was not significant in conditions where the correction was not performed for the difference in this variable in the baseline phase,

the difference in the seventh day (the last measurement of this outcome) was significant due to the difference in this variable between the two groups in the baseline phase. Meanwhile, the effect size for SMD between the two groups for this quantitative outcome was 0.74 (95% CI: 0.22–1.26), indicating a moderate efficacy for this intervention.

The distribution of the three primary binary outcomes under consideration is summarized in Table 4. In addition, the risk ratio, as the indicator for the effect size of binary outcomes, is presented in this Table in the output of the log-binomial regression for both univariate (crude) and adjusted analyses, after controlling the effect of hypertension, diabetes, cardiovascular disease, and kidney and liver diseases. Totally, in the control group, four deaths or hospitalization in ICU, and, in the intervention group, one cases of composite outcome were observed. Although the difference between these frequencies is not statistically significant, based on RR obtained by the statistical model, the efficacy of the intervention in the univariate model was 0.25 (95% CI: 0.03–2.11). After adjusting for hypertension, diabetes, cardiovascular disease, and kidney and liver

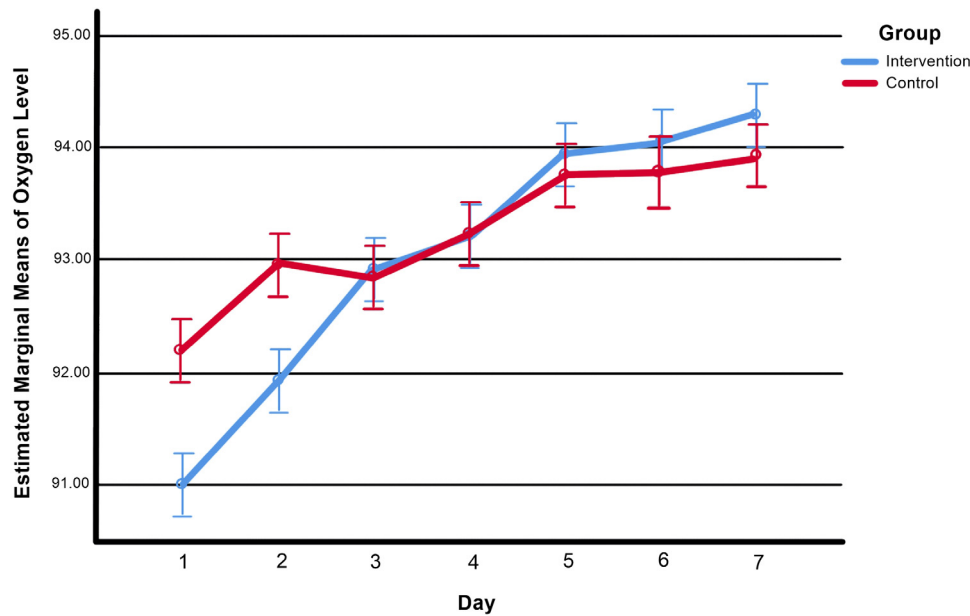


Figure 2. Comparison of the oxygen saturation changes in the intervention and control groups during the 7 d study duration. One-way ANOVA/ANCOVA was used to compare the O₂ saturation level between intervention and comparison groups. The mean difference (MD) and standardized mean difference (SMD) were considered as the effect size indicators. MD (the mean difference of this outcome between intervention and comparison groups) was 0.98 (95% CI: 0.27–1.68). Although this analysis was not significant in conditions where the correction was not performed for the difference in this variable in the baseline phase, the difference in the seventh day (the last measurement of this outcome) was significant due to the difference in this variable between the two groups in the baseline phase. Meanwhile, the effect size for SMD between the two groups for this quantitative outcome was 0.74 (95% CI: 0.22–1.26), indicating a moderate efficacy for this intervention.

Table 4. Binary outcomes Distribution by study GROUPS in addition to the crude and adjusted Risk Ratio (RR)

Outcome	Levels	Intervention N (%)	Comparison N (%)	<i>p</i> ^a	Crude RR ^b (95% CI)	Adjusted RR ^c (95% CI)
Death or ICU admission	Either vs No	1 (3.3)	4 (13.3)	0.20	0.25 (0.03–2.11)	0.31 (0.03–2.88)
Discharge	Discharge vs no discharge	28 (93.3)	25 (83.3)	0.23	1.12 (0.93–1.35)	1.15 (0.96–1.38)
Duration from day 7–discharge, median	> = 4 d vs. <4 d	11 (36.6)	20 (66.6)	0.045	0.63 (0.35–0.99)	0.58 (0.38–1.06)

^aBased on crude model (only effect of intervention) that calculated based on univariable Log-binomial Regression model

^bThe crude model is shown only intervention effect

^cThe adjusted model is shown intervention effect after controlling previous complications that calculated based on multivariable Log-binomial Regression model.

diseases, this index was changed to 0.31 (95% CI: 0.03–2.88).

Also, regarding the discharge outcome, the patients' state in the intervention group was better compared with the control group (about 93.3% against 83.3%). This difference was not also statistically significant. The value of RR for this outcome in the univariate analysis was 1.12 (95% CI: 0.93–1.35), while it was increased to 1.15 (95% CI: 0.96–1.38) in the adjusted analysis. Findings indicated that long hospitalization from day 7 to discharge (four days or above) was 36.6% in the intervention group and 66.6% in the control group. This difference was statistically significant. The effect size indicator of RR was 0.63 (95% CI:

0.35–0.99) in the univariate analysis and reduced to 0.58 (95% CI: 0.38 to 1.06) in the adjusted analysis.

Discussion

With the duration of While the COVID-19 pandemic is getting prolonged, it is necessary to perform studies aiming to achieve a more comprehensive understanding of the COVID-19 pathogenesis because such information can be helpful in developing more effective medications and reducing mortality. In some patients, severe inflammation, cytokine storm, and severely disrupted immune responses may lead to ARDS, ALI, or death (17). Considering the

need for saving time and costs in the current situation caused by the COVID-19 pandemic, drug repurposing is very valuable compared to the development or discovery of new drugs (18). Thus, several clinical trials have been conducted on various drugs and therapies that modulate the immune system (19,20).

Among all these drugs, NLRP3 Inflammasome inhibitors have been investigated in many studies, yielding promising results (21). For example, a clinical trial found that colchicine, as an adjuvant medication along with the standard treatment, could shorten the length of treatment and improve the clinical condition of the patients with COVID-19 (TrialTroveID-379443). Another inhibitor of the NLRP3 inflammasome, low-dose melatonin was investigated by some clinical trials as a potential treatment for COVID-19 (NCT04409522), leading to improvements in the clinical condition and plain radiographs of the patients (22). Recently, in another study, we showed that quercetin (NLRP3 inhibitor) is effective in lowering the serum levels of q-CRP, and LDH as critical markers involved in COVID-19 severity (23). Other NLRP3 inflammasome inhibitors and immunomodulators investigated in the clinical trials as potential COVID-19 treatments include statins, sirolimus, azithromycin, cyclosporine, oridonin, quercetin, and curcumin (24,25), as well as Tranilast, which was evaluated in the present study.

According to our results, a daily dose of 300 mg Tranilast during the first seven days of hospitalization could improve the clinical symptoms of weakness and fatigue in the patients with severe COVID-19 who are under treatment with antivirals and supportive care. It is worth noting that several studies have reported long-term fatigue in patients with COVID-19. Thus, alleviating these symptoms is of great importance (26). The presence of long-term and severe fatigue, which greatly impacts the patients' quality of life, can be due to the extension of inflammation to the CNS, leading to the cerebral accumulation of pro-inflammatory cytokines (27–29). The effect of Tranilast on this important symptom can be explained by its role in causing a significant reduction in the inflammatory factors and pro-inflammatory cytokines, such as IL-1 and TNF.

Moreover, increased activation of neutrophils and NETosis is a potentially destructive mechanism in the inflammation and thrombosis, which especially occurs in the lungs of the patients with severe COVID-19 and those being infected with some other viral infections leading to pulmonary inflammation, such as influenza (30,31). Interestingly, IL-1, as a product of NLRP3 inflammasome, exacerbates this extensive NETosis (32,33). Several studies have noted the increased levels of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF, in patients with COVID-19 (34–37). NLR, CRP, LDH, and d-dimer also have a diagnostic and predictive role in some inflammatory diseases, including the COVID-19 (38–44). According to our re-

sults, Tranilast could reduce the NLR, CRP, and d-dimer in the intervention group compared to the control group, highlighting its effect on preventing severe and destructive inflammation, especially in the inflammatory phase of the disease. Considering the destruction and necrosis observed due to neutrophils in vascular inflammation, thrombosis, and COVID-19 exacerbation, these findings are potentially beneficial in preventing the disease exacerbation and development of respiratory distress.

There are some hepatic and renal adverse effects reported for the Tranilast in some studies (12). Therefore, we evaluated the levels of Cr, BUN, SGOT, and SGPT in both study groups during the intervention and follow-up and There was no significant difference between the two groups. Moreover, Tranilast could lead to significant reductions in the levels of inflammatory markers in the intervention group compared to the control group. Therefore, in addition to its effect on the disease severity, it could increase the effect of antiviral drugs, leading to clinical improvements with fewer complications and dose-dependent toxicity.

Although this trial included statistically insignificant findings in primary binary outcomes (in two of three primary outcomes), the efficacy of intervention cannot be rejected since the effect size indicator of RR was not in the trivial range in two outcomes based on reduction in deaths or hospitalization in ICU or the number of long hospitalization cases. However, the power of statistical analyses based on binary outcomes is obviously lower than statistical analyses based on continuous quantitative outcomes, and this fact should be considered in the design and implementation of trials that include both continuous quantitative and binary outcomes.

In addition, the findings for the quantitative outcome of O₂ saturation indicated that intervention could create significant effectiveness, and even a moderate efficacy was obtained by the effect size indicator of SMD. The present study had some limitations due to the critical situation in the hospitals and for the healthcare personnel. These limitations include the small sample size and the short duration of follow-up, increasing the possibility of bias. According to the above points, it seems that repeating this trial using the confirmatory approach and preferably designing multicenter trials with higher sample sizes and higher generalizability is an effective solution and to confirm the present study results in the future.

Conclusion

According to the present study results, Tranilast probably was effective on patients with severe COVID-19 as adjuvant therapy in combination with antivirals, supportive care, and other standard therapeutic measures. This medication led to improved symptoms, decreased hospital stay, and suppression of the overproduction of pro-inflammatory

cytokines in the intervention group compared to the control group. In addition, the findings for the quantitative outcome of O₂ saturation indicated that intervention could create significant effectiveness, and even a moderate efficacy was obtained by the effect size indicator of SMD. Although this trial included statistically insignificant findings in ICU Admission and Death, the efficacy of intervention cannot be rejected since the effect size indicator of RR was not in the trivial range in two outcomes based on reduction in deaths or hospitalization in ICU or the number of long hospitalization cases. Thus, as a cost-effective and available drug without short-term complications, this medication is recommended to be further investigated in future studies.

Conflict of Interest

All authors have no conflict of interest to declare.

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