An investigation into the Effects of Intravenous Vitamin C on Pulmonary CT Findings and Clinical Outcomes of Patients with COVID19- Pneumonia A Randomized Clinical Trial

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Purpose: In late December 2019, a series of unexplained cases of pneumonia were reported in Wuhan, China. On January 12, 2020, the World Health Organization temporarily named the virus responsible for the emerging cases of pneumonia as the 2019 coronavirus. Acute respiratory distress syndrome (ARDS) due to Covid-19 has rapidly spread around the world, and while no specific treatment or vaccine has been reported, mortality rates remain high. One of the suggested treatments for cellular damage in the pathogenesis of ARDS caused by the coronavirus is the administration of high doses of intravenous vitamin C. Considering the paucity of literature on the therapeutic effects of high doses of intravenous vitamin C in patients with ARDS resulting from the coronavirus, this study was conducted to assess this therapeutic supplement in these patients.

Materials and Methods: This study was performed as a single-center clinical trial in patients with a documented diagnosis of COVID-19 pneumonia. 54 eligible patients with moderate to severe COVID-19 symptoms, based on specific inclusion and exclusion criteria, were included in the investigation and randomly divided into two groups. The control group consisted of 26 patients who received standard treatment, whereas the treatment group was comprised of 18 patients administered intravenous vitamin C at a dose of 2 g every 6 hours for 5 days in addition to standard treatment. Demographic characteristics, underlying diseases, length of hospital stay, and mortality rates were reviewed and collected. Oxygen saturation, respiratory rates, serum C Reactive Protein (CRP) levels, lymphopenia and lung parenchymal involvement on CT were investigated at the time of admission and on the sixth day after hospitalization.

Results: Of these variables, the amount of oxygen saturation in the vitamin C group increased significantly from 86 ± 5% on the first day of hospitalization to 90 ± 3% on the sixth day of hospitalization (*P* value = 0.02). Also, the respiratory rate in the vitamin C group decreased significantly from 27 ± 3 on the first day of hospitalization to 24 ± 3 on the sixth day of hospitalization (*P* value = 0.03). Lung CT scans of patients in the two groups reported by two radiologists were also compared. Based on the report of the radiologists, the rate of lung involvement in the vitamin C group was significantly lower than in the control group at the end of treatment (*P* value = 0.02).

Conclusion: Due to the effectiveness of high doses of intravenous vitamin C on reducing lung involvement and improving clinical symptoms, further studies with a larger sample size are recommended to demonstrate the effects of this drug supplement.

Keywords: vitaminC; COVID-19; coronavirus; ARDS; treatment

INTRODUCTION

Human respiratory coronaviruses were first recognized in the 1960s and have been known to cause respiratory infections with rather mild symptoms. However, two infamous infectious coronaviruses in the Beta coronavirus genus, the severe acute respiratory syndrome (SARS) virus and Middle East respiratory syndrome coronavirus (MERS-CoV), can cause severe respiratory tract infections with high mortality.(1)

Pathological tests of samples obtained from patients who had died of SARS showed diffuse alveolar lesions, accompanied by prominent hyperplasia of pulmonary epithelial cells and presentation of activated alveolar and interstitial macrophages. Considerably, these pulmonary manifestations were usually found after the release of a cytokines and in the absence of other opportunistic infections. Therefore, local inflammatory responses could result in alveolar damage.(2)
Supernumerary macrophages produced high levels of

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Table 1. Stages of lung involvement in COVID-19

Stage	Definition
1 (Early)	Chest CT shows single or multiple scattered patchy or conglomerate ground-glass opacities, predominantly in the middle and lower lungs along with bronchovascular bundles. These ground glass lesions are often located in the peripheral and subpleural areas of the lung. Intra- and interlobular septal thickening, sometimes present in the areas of ground-glass opacity, can give a crazy-paving pattern.
2 (Advanced)	Chest CTs show new lesions that are similar to the earlier lesions described above. Also, findings from the early stage of disease increase in density and extent, coexisting with the new areas of disease. As areas of consolidation grow, air bronchograms are often present in the areas of consolidation.
3 (Severe)	Chest CT shows diffuse consolidation of the lungs of varying density secondary to the fibrous exudate into the alveolar cavity, air bronchograms and bronchial dilation. Nonconsolidated areas of the lung appear as patchy ground-glass opacity. When most of the lungs are involved, the lungs appear as a "whited out a lung." The pleura is thickened and there can be a small amount of pleural effusion.
4 (Dissipation)	The images show gradual resolution of the ground glass opacity and consolidation in the lungs with some residual curvilinear opacities compatible with fibrosis.

inflammatory mediators through type I IFN stimulation. Moreover, type I IFN-induced immune dysregulation led to the apoptosis of T cells, which would normally promote virus clearance, resulting in reduced numbers of virus-specific CD8 and CD4 T cells. Overall, massive repletion of pathogenic inflammatory macrophages increased the severity of SARS. (3) Numerous studies have shown that vitamin C plays an important role in various aspects of the immune system, especially the function of immune cells.[4,5]

Vitamin C (ascorbic acid) is a powerful antioxidant that helps the immune system and supports several intrinsic immune cell functions and adaptive immune systems. It forms an epithelial barrier against pathogens and eliminates oxidants in the skin, thus protecting them from environmental oxidative stress. Vitamin C rapidly donates electrons, which disrupts the damage of oxidative biomolecules.[6] It is also a cofactor for various enzymes, such as the monooxygenase and the dioxygenase enzymes.

Individuals with vitamin C deficiency are more prone to fatal infections such as pneumonia. In turn, Infections can affect vitamin C levels due to increased inflammation and metabolic needs. Respiratory infection is particularly serious in individuals who are already malnourished.[7]

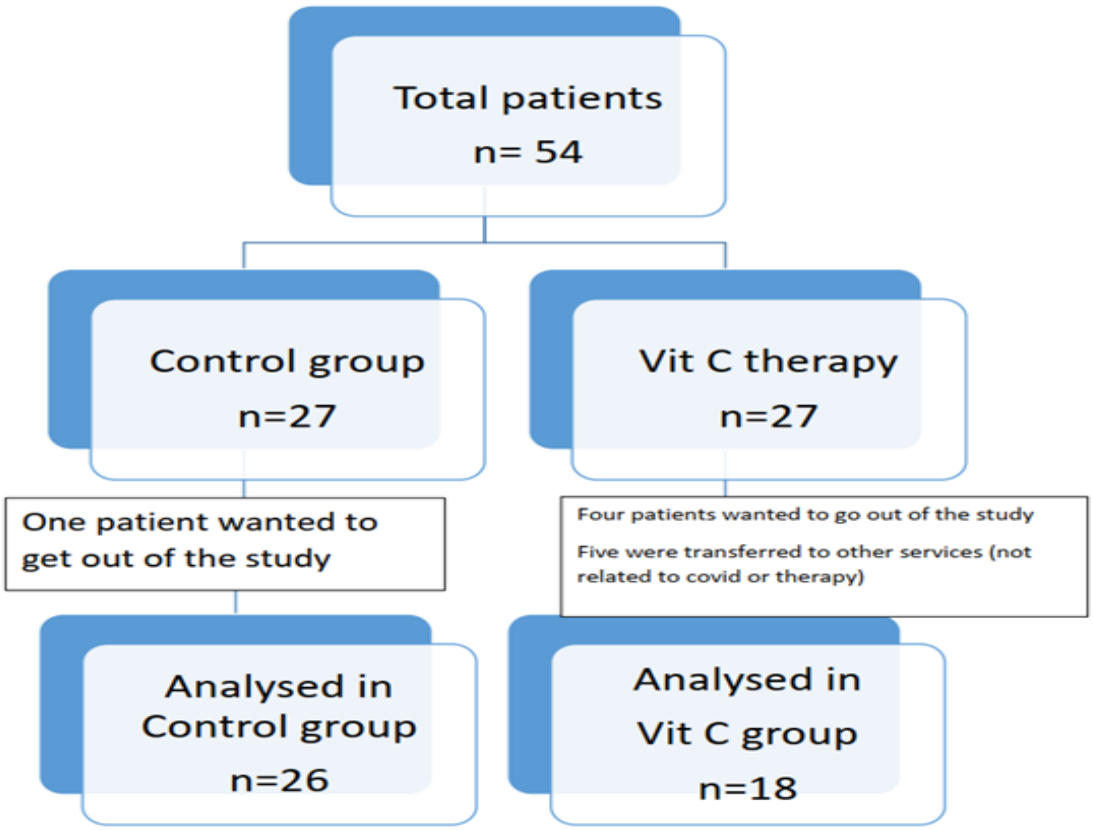

Figure 1. Patients enrollment diagram

Table 2. For classifying lung zone involvement, three-zone were defined as follows: upper zone: above the carina region, middle zone: the area between the carina and inferior pulmonary vein, and lower zone: below the inferior pulmonary vein

Lung involvement scoring	Definition
0	None
1	Mild (involvement of 2 zone)
2	Moderate (involvement of 4 zone)
3	Severe (involvement of 6 zone)

Based on previous experiences with the use of intravenous vitamin C in critically ill patients and patients with respiratory infections, due to the high morbidity and mortality of COVID-19 pneumonia, we decided to study the possible effect of high dose intravenous vitamin C in COVID-19 pneumonia.

MATERIALS AND METHODS

This study was performed as a single-center clinical trial for patients with a confirmed diagnosis of COVID-19 pneumonia from March to May 2020 at the Referral Center of Shahid Labbafi Nejad Hospital in Tehran. The present clinical trial protocol has been approved by the Iranian Registry of Clinical Trials (IRCT ID: IRCT20211004052664N1).

The inclusion criteria in our study were as follows: age ≥ 18y, hospitalized patients with: Respiratory rate >30/min or oxygen saturation <93% and pulmonary infiltration> 50%); PCR confirmation for the nuclide acid of SARS-COV-2 in a Nasopharyngeal swab specimen and chest lung CT scan compatible with COVID-19 patterns. The Exclusion criteria were the following: known allergic reaction to vitamin C, shortness of breath due to cardiogenic pulmonary edema, pregnancy or breastfeeding, chronic renal failure, diabetic ketoacidosis and a history of nephrolithiasis.

Sample size was based on a pilot study assuming the incidence of fibrosis to be around 50% in no vitamin C regimen while near 15% in vitamin C therapy group. Considering a confidence interval of 95% with a power of 80%, 25 patients were required in each arm of this study. Thinking of some drop offs 27 patients were enrolled in each group.

Fifty-four patients were enrolled in this study. They were randomized through a computerized random allocation of patients. One patient in the control group and nine in the study arm were excluded as depicted in the consort chart. Finally, all variables were analysed using SPSS 23. Normality test of Kolmogorov-Smirnov was done and after confirmation of non-skewed data and absence of any outlier, parametric statistics were applied. Independent t-test was utilized for means while Chi-two for frequencies. A p-value of 0.05 was considered as statistically significant and Confidence Intervals of 99% are mentioned as required

Treatment Design

Study variables such as age and underlying diseases were selected similarly to minimize the distorting effects of these variables. The control group (group A) consisted of 26 patients who received standard treatment [Hydroxicholoroquine (400 mg stat) and Kaletra (400/100 mg q 12 h) and Interferon beta-1a (44 micrograms three times)] and the treatment group (group B) included 18 patients receiving intravenous vitamin C at a dose of 2 g every 6 hours for 5 days in addition to standard treatment.

Demographic characteristics, underlying diseases, length of hospital stay and mortality rates were reviewed and collected. Oxygen saturation, respiratory rates, serum CRP levels, lymphopenia, lung parenchymal involvement on CT at the time of admission and on the sixth day after hospitalization were investigated. In three cases in the vitamin C treatment group, the control CT scan was not performed on the sixth day due to instability of vital signs. The patient's lung CT scans were examined by two experienced radiologists who had no knowledge of the patients' groups. Both radiologists reported lung CT scans based on the pattern and extent of lung involvement. (**Tables 1, 2**)

This study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences in Tehran, Iran. (IR.SBMU.RETECH.REC.1399.067)

Table 3. Demographic Characteristics, admission-time clinical and laboratory findings in the two groups.

	Vitamin C group N=18	Control group N=26	*P* value
Age (year)	58 ± 19	61 ± 17	0.73
Gender (M/F)	8/10	18/8	0.14
Start of symptom to admission (day)	9 ± 6	7 ± 4	0.39
Diabetes	33%	35%	1
Hypertension	33%	46%	0.48
Ischemic heart disease	27%	19%	0.67
Chronic Kidney Disease	0	15%	0.23
Chronic Lung Disease	7%	8%	1
Immunocompromised	20%	8%	0.22
Respiratory rate	27 ± 3	29 ± 2	0.61
O2 Sat (%)	86 ± 5	87 ± 2	0.23
WBC	9380 ± 5113	7253 ± 3936	0.19
Lymphocyte	2233 ± 1970	1044 ± 362	0.032
Neutrophils	6820 ± 3840	5881 ± 3600	0.43
PLT	257 ± 86	218 ± 75	0.13
CRP	42 ± 17	35 ± 31	0.49
Pre-treatment CT Stage			
I	33%	23%	0.51
99%CI:0.49-0.52			
II	13%	27%	
III	40%	46%	
IV	14%	4%	

Table 4. Day-6 clinical and laboratory findings, mortality and morbidity rates and length of stay in the two groups.

	Vitamin C group N=18	Control group N=26	*P* value
Day-6 Respiratory rate	24 ± 3	28 ± 4	0.028
Day-6 O2 sat (%)	90 ± 3	87 ± 5	0.021
Day-6 WBC	6900 ± 2484	7788 ± 5193	0.53
Day-6 Lymphocyte	1706 ± 1811	1186 ± 696	0.21
Day-6 Neutrophil	4878 ± 1652	6349 ± 4657	0.18
Day-6 Plt	272 ± 68	228 ± 86	0.092
Day-6 CRP	29 ± 15	29 ± 22	0.52
On Recigen Treatment	33%	42%	0.67
Duration of Hospitalization (day)	14 ± 8	17±8	0.23
Needed intubation			
Death (patients)	0	4	0.21
Post Treatment CT Stage			
I	13%	4%	0.71
99%CI:0.70-0.72			
II	7%	4%	
III	20%	31%	
IV	60%	61%	
Post treatment Fibrosis in CT			
0	7%	35%	0.023
99%CI: 0.021-0.029			
1	40%	8%	
2	33%	23%	
3	20%	35%	

RESULTS

In this study, twenty-six patients were enrolled as a control group, while eighteen received vitamin C as a treatment group. The average age of the control group was 61 years, with 18 women and 8 men. In this group, 35% had diabetes, 46% had hypertension, 19% had ischemic heart disease, 15% had chronic kidney disease, 8% had chronic lung disease, and 8% had received immunosuppressive medication. In the intervention group, the mean age was 58 years, of which 10 were women and 8 were men. In this group, 33% had diabetes, 33% had hypertension, 27% had ischemic heart disease, 0% had chronic kidney disease, and 7% had chronic lung disease, and 20% had been administered immunosuppressive drugs.

The oxygen saturation and respiratory rate, leukocyte, lymphocyte, neutrophil and platelet counts, CRP levels on the first and sixth days of hospitalization, the length of hospital stay and mortality rates were compared between the two groups (**Tables 3 and 4**). Of these variables, the amount of oxygen saturation in the vitamin C group increased significantly from 86 ± 5% on the first day of hospitalization to 90 ± 3% on the sixth day of hospitalization (*P*-value = 0.02). Also, the respiratory rate in the vitamin C group decreased significantly from 27 ± 3 on the first day of hospitalization to 24 ± 3 on the sixth day of hospitalization (*P*-value = 0.03).

Lung CT scans of patients in the two groups reported by two radiologists were also compared. Based on the reports of the radiologists, the rate of improvement in lung involvement at the end of treatment was significantly higher in the vitamin C group in comparison to the control group. (*P*-value = 0.02).

DISCUSSION

This study was conducted to shed more light on the effects of vitamin C on the clinical symptoms, laboratory findings and pattern and extent of lung involvement on

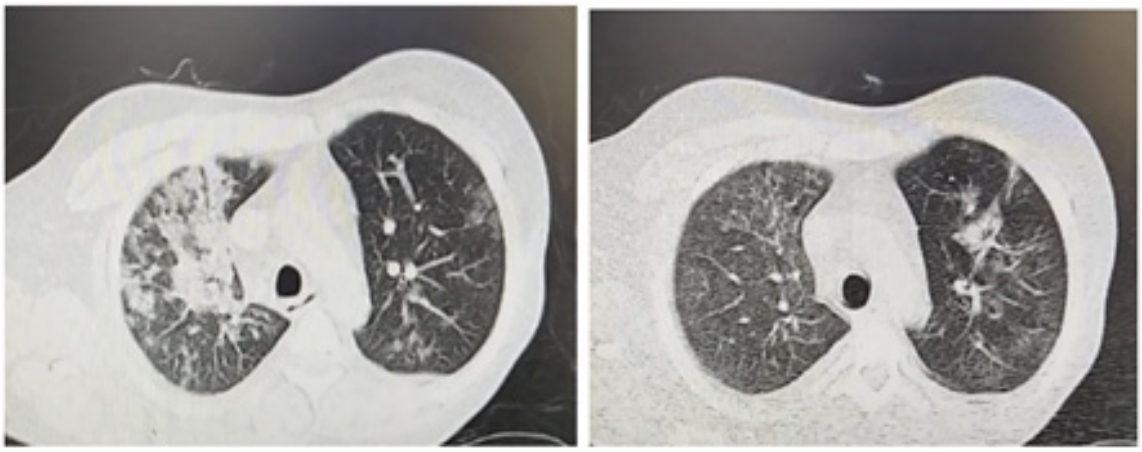

Figure 2. A 63-year-old patient who no history of underlying disease. He was recently hospitalized with complaints of cough and dyspnea and diagnosed with COVID 19 pneumonia. Left image: The first day of admission. Consolidation and peribronchovascular thickening in the right upper lobe. Right image: On the sixth day of treatment with vitamin c. Mottled ground glass infiltration and fine reticulation is present in the right upper lobe.

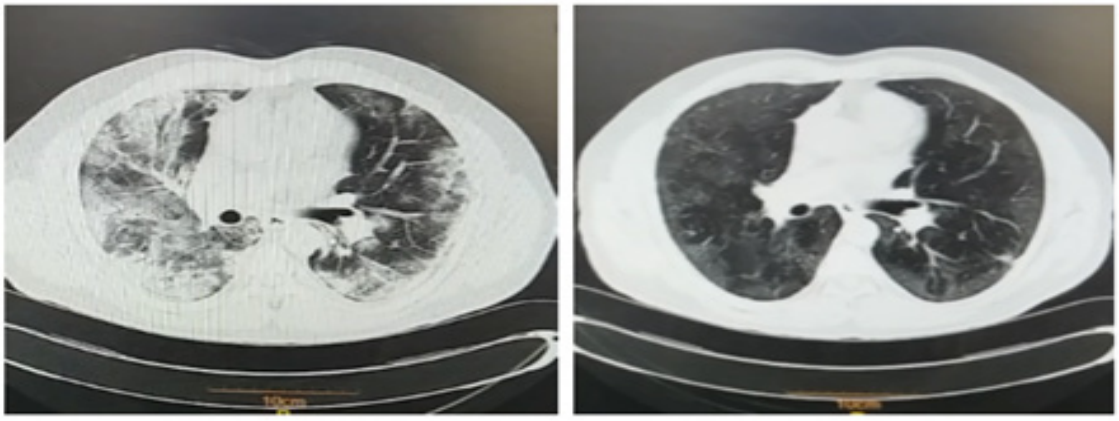

Figure 3. A 42-year-old patient who had no history of underlying disease. He was hospitalized with complaints of cough and dyspnea and diagnosed with COVID 19 pneumonia. Left image: The first day of admission. Mixed consolidation and ground glass with peripheral and peribronchovascular distribution. Right image: One month after treatment with vitamin C. Only mottled subpleural ground-glass infiltrations are observed.

CT in patients with COVID-19.

One hypothesis about the mechanisms by which COVID-19 can cause severe forms of the disease is the occurrence of an uncontrolled inflammatory response in the course of the disease.[8]. An important point in the pathophysiology of vitamin C (ascorbic acid) is the regulation of cytokine storms and the reduction of oxidative damage in the endothelium, which in some studies is valuable in controlling the severe form of Covid 19 diseases.[9 ,10]

Various results have been reported in the clinical outcome of patients with severe sepsis and acute respiratory distress syndrome following high-dose intravenous vitamin C administration.[11–13].

In a study conducted by Hossaini Zabet et al in 2016 in Iran, 28 patients with septic shock and ARDS syndrome were injected with vitamin C at a dose of 25 mg /kg body weight daily for three days. The variables of this study included hemodynamic parameters, the oxygenation status, Laboratory parameters, the need for vasopressors and the mortality rates of the patients. The need for vasopressors and mortality rates in this group were significantly reduced compared to the control group, but there was no significant difference in the length of stay in ICU. Also, there was no significant difference between the two groups in the other variables. [14]

In our study no significant differences were observed between the two groups in terms of age, sex and underlying comorbidities. Our results revealed that the group administered vitamin C demonstrated considerably lower respiratory rates on the sixth day compared to the control group (*P* value = 0.03).

It is noteworthy that in our study, there was a clear improvement in blood oxygen levels and respiratory rate on the sixth day in the group receiving vitamin C, which was statistically significant. (*P* value = 0.02).

In a 2019 study by Zhi Yong Peng in China, 85% of the 252 patients with COVID 19 who received vitamin C (at a dose of 1 gram per hour for six hours and then 3 grams daily) showed improvement of disease symptoms. However, in patients with sepsis and ARDS syndrome who were treated with high-dose vitamin C, there was no significant improvement in clinical symptoms and prognosis. All things considered, due to the different results, it was recommended to conduct more studies on the effects of vitamin C in the treatment of patients with COVID 19. In this study, the effects of vitamin C on patients' lab tests and lung CT scans were not investigated.[15]

At an RCT in China, 56 patients with severe SARS-CoV-2 pneumonia were studied with a high dose of intravenous vitamin C (12 g every 12 hours) for 7 days. Finally, in the group receiving vitamin C, an increase in PaO2 / FiO2 and lower levels of IL-6 were reported on day 7 compared to the control group.[16]

In our study, we did not identify a statistically significant difference in terms of laboratory findings such as day-6 lymphocyte counts, Neutrophil counts, serum CRP levels, and mortality rates between the two groups. It is notable that, although the length of hospital stay between the two groups was not significantly different, the number of hospitalization days for patients with vitamin C was lower. It is possible to get better results by increasing the sample size.

In the study of Jamali Moghadam et al., The effect of a high dose of intravenous vitamin C (6 g daily) on 30 patients with Covid-19 pneumonia was investigated. The rate of fever and oxygen saturation on the third day of treatment and the duration of hospitalization was significantly better compared to the control group, but the period of hospitalization in the ICU and the mortality rate were not significantly different from the control group, which is similar to the results of the present study.[17]

In a study by HakamiFard et al., A low dose of vitamin C (1000 mg daily) was evaluated in 38 patients with non-severe Covid-19 pneumonia. There was no significant difference in response to treatment, length of hospital stay, and mortality compared with the control group.[18]

According to the two experienced radiologists' reports, the stages of lung involvement did not change considerably in the two groups before and after treatment. (*P* value = 0.6) Lung involvement scoring in the control group after treatment was: 35% none, 8% mild, 23%

moderate, and 35% severe. While lung involvement scoring in the vitamin C group after treatment was: 7% none, 40% mild, 33% moderate, and 20% severe. This difference between the two groups was significant. (*P* value = 0.02).

This study had certain limitations. The interval between the onset of symptoms and the patients' time of hospitalization varied between patients and this factor might have affected all variables in the study. It is generally assumed that the earlier patients are admitted to hospital, the slower the progression of the disease and the better the response to treatment. Due to limited access to intravenous vitamin C in Iran, the sample size was relatively small, which could in turn have had an impact on the reliability of the study. To better evaluate the effects of vitamin C on lung fibrosis, a lung CT scan carried out 4 to 6 weeks after the onset of the disease would have been beneficial, but performing these scans was not possible due to lack of cooperation by patients. Considering the high prevalence of pneumonia and acute respiratory distress syndrome caused by the coronavirus in Iran and other countries and the mortality rate of this disease, It is necessary to identify effective treatment methods. Also, Bearing in mind the complications of this disease such as lung fibrosis, the use of treatments that prevent lung fibrosis and improve lung function in patients is of the utmost importance. Due to the effectiveness of high doses of intravenous vitamin C in this study on reducing lung involvement and improving clinical symptoms, further studies with a larger sample size are recommended to demonstrate the effects of this drug supplement.

CONCLUSIONS

This review aimed to evaluate the effect of vitamin C treatment in patients With COVID-19 pneumonia. In this study, we found that there were improvements in peripheral oxygen saturation and the respiratory rate in the group who were treated with high-dose vitamin C.

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CONFLICT OF INTEREST

The authors did not declare any conflict of interest.

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