Original Article

The efficacy of hydrogen/oxygen therapy favored the recovery of omicron SARS-CoV-2 variant infection: results of a multicenter, randomized, controlled trial

Meng-Meng Shi,[1,2,3,†] Yun-Tian Chen,[4,†] Xiao-Dan Wang,[5,†] Yun-Feng Zhang,[6,†] Ting Cheng,[1,2,3,†] Hui Chen,[7] Feng Sun,[8] Hong Bao,[9] Rong Chen,[1,2,3] Wei-Ning Xiong,[4,*] Yuan-Lin Song,[5,*] Qing-Yun Li,[1,2,3,*] and Jie-Ming Qu[1,2,3,*]

[1]Department of Respiratory and Critical Care Medicine, Ruijin Hospital and [2]Institute of Respiratory Diseases, Shanghai Jiao Tong University School of Medicine, 197 Rui Jin Er Road, Shanghai, 200025, China
[3]Shanghai Key Laboratory of Emergency Prevention, Diagnosis and Treatment of Respiratory Infectious Diseases, 197 Rui Jin Er Road, Shanghai 200025, China
[4]Department of Respiratory and Critical Care Medicine, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhizaoju Lu, Shanghai 200011, China
[5]Department of Respiratory and Critical Care Medicine, Zhongshan Hospital, Fudan University, 180 Fenglin Road, Shanghai 200032, China
[6]Department of Respiratory and Critical Care Medicine, Shanghai Putuo District Liqun Hospital, 910 Taopu Road, Shanghai 200333, China
[7]Department of Anesthesiology and Perioperative Medicine, Shanghai Fourth People's Hospital, School of Medicine, Tongji University, 1279 Sanmen Road, Shanghai 200000, China
[8]Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, National Medical Center for Infectious Diseases, Huashan Hospital, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, China
[9]Department of Respiratory and Critical Care Medicine, Shanghai Pudong Hospital, 2800 Gongwei Road, Shanghai 201399, China

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Clinical studies had found that hydrogen/oxygen mixed inhalation was beneficial to ameliorate the respiratory symptoms in the adjuvant treatment of patients with COVID-19. We aimed to explore the efficacy of hydrogen/oxygen therapy in favoring the recovery of Omicron SARS-CoV-2 variant infection. There were 64 patients who randomly assigned to receive hydrogen/oxygen inhalation (32 patients) and oxygen inhalation (32 patients). The average shedding duration of Omicron in hydrogen/oxygen group was shorter than oxygen group. The trend of cumulative negative conversion rate of Omicron increased gradually after the third day. The IL-6 levels in hydrogen/oxygen group decreased by 22.8% compared with the baseline. After hydrogen/oxygen mixed gas inhalation, the lymphocyte count increased to 61.1% of the baseline on the 3rd day in the hydrogen/oxygen group. More patients in the hydrogen/oxygen group had resolution of pulmonary lesions. Our study showed the beneficial trends of molecular hydrogen in treating patients with COVID-19, which may offer a prospective solution to adjuvant therapy for COVID-19 Patients.

***Key Words*: hydrogen/oxygen therapy, SARS-CoV-2, Omicron variant, clinical trial**

During the past three years, coronavirus disease 2019 (COVID-19) by infection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a major global public health challenge.[1–4] World Health Organization (WHO) has proposed five "variants of concern" (VOCs) of SARS-CoV-2, including Alpha, Beta, Gamma, Delta, and Omicron. Since December of 2021, Omicron variant has become the main epidemic strain with high transmissibility. As effective antioxidant agent, hydrogen has been emphasized from the beginning of 2000s.[5] It is safe to inhale hydrogen and oxygen mixed gas according to the experience of divers for many years. Studies on hydrogen/oxygen mixed gas inhalation as the adjuvant treatment of COPD and tracheal stenosis also showed that inhalation of low-density gas could improve airway resistance, increase oxygen diffusion and oxygen flow, and improve dyspnea. In 2020, a retrospective study (NCT 04378712) found that hydrogen/oxygen mixed inhalation is beneficial to ameliorate the respiratory symptoms in the adjuvant treatment of patients with COVID-19.[6,7] However, it is still unknown whether it is effective in the patients with Omicron infection. Thus, we conducted a prospective, multicenter, randomized study to explore the efficacy and safety of hydrogen/oxygen mixed gas inhalation in the adjuvant treatment of COVID-19.

Methods

Study design and participants. A total of 64 patients with COVID-19 hospitalized in seven hospitals were enrolled in the study (between May 16, 2022 and June 15, 2022). The study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine (No. 2022-84), and registered on www.clinicaltrials.gov, No. NCT05532852. The Hydrogen/Oxygen Generators (model AMS-H-03) were provided by Shanghai Asclepius Meditec Co., Ltd., China. Patients were randomly assigned to hydrogen/oxygen group (32 patients) and oxygen group (32 patients) by random envelope. On the basis of standard-of-care, patients in hydrogen/oxygen group received hydrogen/oxygen mixed gas inhalation at 3 L/min via nasal cannula (at least 7 h per day), while those in oxygen group received oxygen therapy alone (Fig. 1). The clinical symptoms and signs, laboratory examinations and chest scan images were recorded on day 1, the day 3 and the day 5.

Inclusion criteria. (1) Ages from 18 years old to 80 years old. (2) Common type of COVID-19 severity, according to the ninth version of the guidelines on the Diagnosis and Treatment of COVID-19 by the National Health Commission. (3) The subjects or their family members voluntarily participated in the study and

[†]Equally contributed to this work.
*To whom correspondence should be addressed.
E-mail: xiongdoctor@qq.com (W-NX), ylsong@163.com (Y-LS), liqingyun68@hotmail.com (Q-YL), jmqu0906@163.com (J-MQ)

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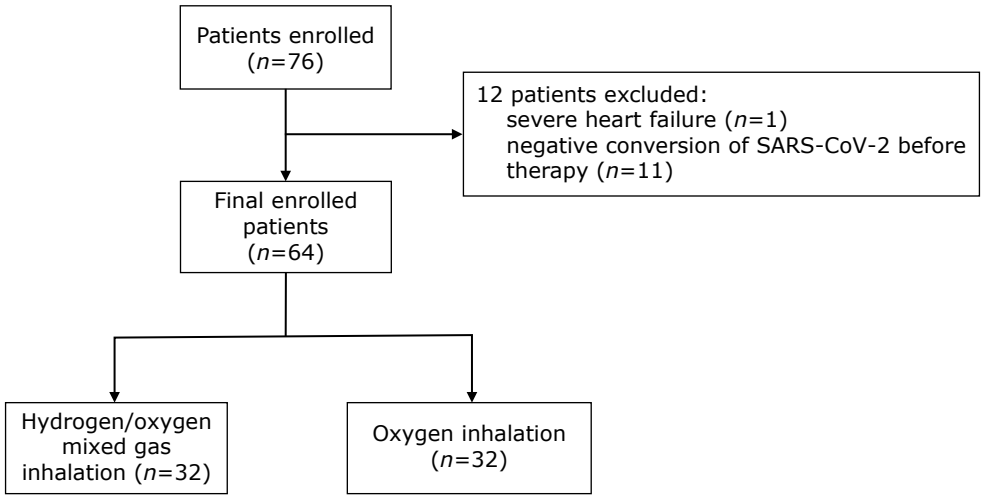

Fig. 1. Flow diagram of the study.

signed the informed consent.

Exclusion criteria. (1) Subjects whose COVID-19 severity as mild, severe or critical, as well as the recessive infection. (2) Suffering from malignant tumors who were current treated. (3) Known to be intolerant to inhalation therapy. (4) Subjects who are known to be unable to sign informed consent due to mental disorder or cognitive impairment. (5) Those with immune deficiency, using corticosteroids or other immunosuppressants. (6) Severe heart, liver or kidney failure, Acute exacerbation of chronic obstructive pulmonary disease, Acute attack of asthma. (7) Those who use non expectorant and antioxidant drugs, including large doses of vitamin C and vitamin E. (8) Subjects participating other clinical trials. (9) Pregnancy. (10) According to the judgment of the researcher, the one who are not suitable to participate in this study.

Outcomes and assessments. The primary outcomes of this study were the shedding duration of SARS-CoV-2. The secondary outcomes included negative conversion rate of SARS-CoV-2, levels of inflammatory factors [C-reactive protein (CRP), interleukin 6 (IL-6), lymphocytes count, etc.] and improvement of CT images.

Statistical analysis. Analyses of the full-analysis set were performed with SAS software ver. 9.4. Count (percentage) was adopted for summarizing categorical variables and compared with *Chi*-square tests or Fisher's exact tests. Continuous variables were presented with mean ± SD and compared with independent *t* test or Wilcoxon rank-sum test. Time-to-event analyses were performed using the Kaplan–Meier method for the medians, the log-rank test and hazard ratio (HR) along with the 95% confidence interval (95% CI) were calculated to reflect the difference of the event between groups. All testing was two-sided, with $p<0.05$ being statistically significant.

Results

Clinical characteristics of participants. Total of 64 patients were included, including 32 in the hydrogen/oxygen group (23 males, 9 females), 32 in the oxygen group (12 males, 20 females). The average age was 63.9 ± 8.85 years in the oxygen group, and 61.8 ± 12.72 years in the hydrogen/oxygen group. 21.9% (7/32) of the oxygen group vs 34.4% (11/32) of the hydrogen/oxygen group had a history of smoking. In the oxygen group, 56.3% (18/32) of the patients had comorbidities, including malignant tumors (6.3%), cardiovascular diseases (15/32, of which 7 were hypertension), and there were no respiratory diseases, chronic liver diseases, chronic kidney diseases, immune deficiencies, and other comorbidities. In the hydrogen/oxygen group, 50% (16/32) of the patients had comorbidities, including malignant tumors (12.5%), cardiovascular diseases (10/32, of which 7 were hypertension), one patient had respiratory diseases, and one patient had immune deficiency. No additional comorbidities such as chronic liver disease and chronic kidney disease were reported (Table 1).

Among the 64 patients, 39.1% (25/64) were received Paxlovid (17 cases in the oxygen group vs 8 in the hydrogen/oxygen group), 25% (16/64) of the patients used Lianhua Qingwen granule (11 in the oxygen group vs 5 patients in the hydrogen/oxygen group). Five patients accepted the antibiotic treatment in the hydrogen/oxygen group, meropenem ($n = 1$), moxifloxacin ($n = 1$), cefdinir ($n = 1$), ceftriaxone ($n = 1$) and cefoperazone/sulbactam ($n = 1$). Two patients in the oxygen group were combined with antibiotics, i.e., one with levofloxacin, and one with cefixime (Supplemental Table 1*).

Symptoms of the patients. The main symptom of the patients before enrollment was cough (68.8% in the oxygen group and 56.3% in the hydrogen/oxygen group), followed by expectoration (25.0% in the oxygen group and 28.1% in the hydrogen/oxygen group). Other symptoms were reported in low frequency, such as fever, fatigue, shortness of breath, dyspnea, and chest pain (Table 2).

Changes of clinical manifestations, laboratory and radiological findings.

The effect of hydrogen/oxygen inhalation on shedding duration of variant Omicron. The average shedding duration of Omicron in hydrogen/oxygen group was shorter than oxygen group (3.3 ± 2.30 days vs 4.1 ± 3.12 days, $p = 0.374$). At the first 5 days, 28 patients (87.5%) in the hydrogen/oxygen group and 24 patients (75%) in the oxygen group showed negative conversion of Omicron. By 10 days, all (100%) patients in hydrogen/oxygen group (32/32) eliminated the virus, while as 93.8% (30/32) in the oxygen group ($p = 0.492$). We found that the trend of cumulative negative conversion rate of Omicron increased gradually after the third day compared with the oxygen group (Fig. 2, $p = 0.283$).

The effect of hydrogen/oxygen inhalation on inflammatory indicators. In terms of inflammatory indicators, The IL-6 levels in hydrogen/oxygen group decreased by 22.8% compared with the baseline, while which of the oxygen group slightly increased on the 3rd day. On the 5th day, the IL-6 levels continuously decreased by 20.1% vs 13.1% in oxygen group (Fig. 3A). Although the descending change of CRP from baseline in

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Table 1. Demographic information and baseline characteristics of patients

	Oxygen group (n = 32)	Hydrogen/oxygen group (n = 32)	p values
Age (years)	63.9 (8.85)	61.8 (12.72)	0.979
Sex			
Male	12 (37.5%)	23 (71.9%)	0.006
Female	20 (62.5%)	9 (28.1%)	
Height (cm)	162.6 (7.50)	168.1 (8.44)	0.008
Weight (kg)	61.80 (13.73)	67.02 (14.18)	0.146
Smoking history	7 (21.9%)	11 (34.4%)	0.266
Comorbidities	18 (56.3%)	16 (50.0%)	0.449
Malignant tumors	2 (6.3%)	4 (12.5%)	0.395
Cardiovascular diseases and hypertension	15 (46.9%)	10 (31.3%)	0.238
Chronic lung diseases	0 (0.0%)	1 (3.1%)	0.474
Chronic liver diseases	0 (0.0%)	0 (0%)	NA
Chronic kidney diseases	0 (0.0%)	0 (0%)	NA
Immune deficiencies	0 (0.0%)	1 (3.1%)	0.474
Mental disorders	0 (0.0%)	1 (3.1%)	0.474

Table 2. Symptoms at enrollment

	Oxygen group (n = 32)	Hydrogen/oxygen group (n = 32)	p values
Main symptoms	26 (81.3%)	19 (59.4%)	0.055
Fever	1 (3.1%)	1 (3.1%)	1
Fatigue	5 (15.6%)	7 (21.9%)	0.522
Cough	22 (68.8%)	18 (56.3%)	0.302
Expectoration	8 (25.0%)	9 (28.1%)	0.777
Shortness of breath	6 (18.8%)	0 (0.0%)	0.024
Dyspnea	3 (9.4%)	0 (0.0%)	0.238
Chest tightness	6 (18.8%)	1 (3.1%)	0.104
Chest pain	1 (3.1%)	0 (0.0%)	1

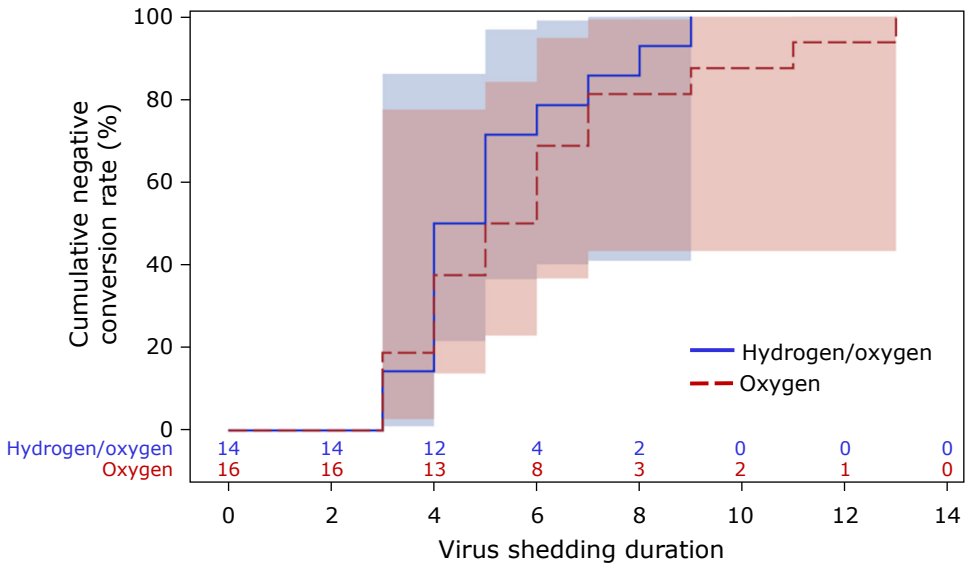

Fig. 2. Analysis of the virus shedding duration.

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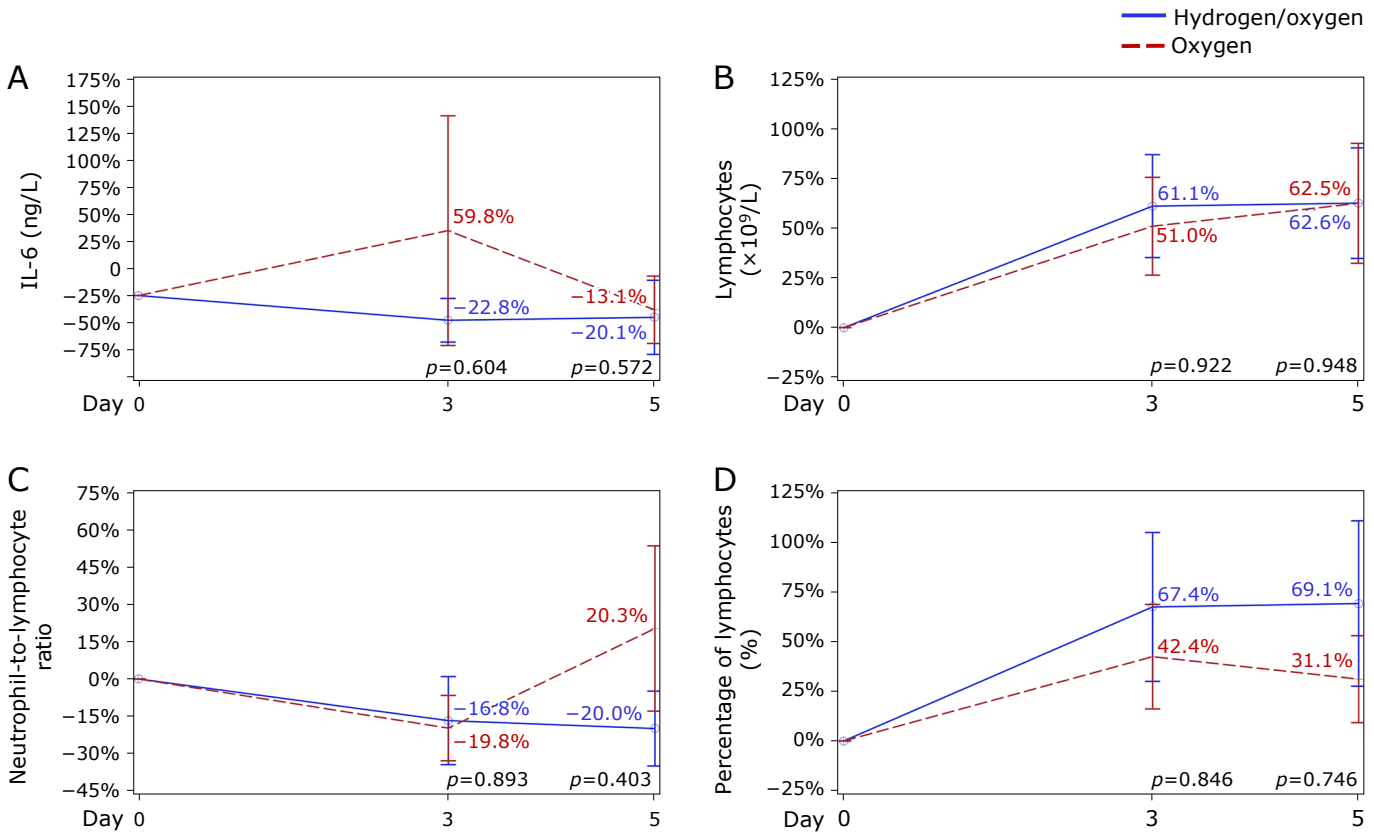

Fig. 3. The changes of inflammatory indicator. (A) IL-6 levels, (B) lymphocyte count, (C) neutrophil-to-lymphocyte ratio, (D) lymphocyte percentage.

hydrogen/oxygen group was smaller than that in the control group, the CRP levels in the treatment group decreased more significantly than that in the control group (Supplemental Fig. 1*).

After hydrogen/oxygen mixed gas inhalation, the lymphocyte count increased to 61.1% of the baseline on the 3rd day and continued to increase to 62.6% on the 5th day, and the lymphocyte count in the oxygen group increased less than that in the hydrogen/oxygen group (Fig. 3B). In the hydrogen/oxygen group, the lymphocyte percentage ascended to 67.4% of the baseline on the 3rd day and continued to rise to 69.1% on the 5th day. In the oxygen group, the lymphocyte percentage rose to 42.4% on the 3rd day, but fell back to 31.1% on the 5th day (Fig. 3D).

We further observed the neutrophil-to-lymphocyte ratio and found that there was no significant difference between the hydrogen/oxygen group and the oxygen group on the third day (16.8% vs 19.8%). However, the ratio in the hydrogen/oxygen group continued to decline to 20.0% of the baseline, while it increased to 20.3% of the baseline in the oxygen group (Fig. 3C).

The effect of hydrogen/oxygen inhalation on pulmonary lesions. The degrees of resolution of pulmonary lesions after hydrogen/oxygen mixed gas inhalation were observed at the 7th day among all the patients. Levels of pulmonary lesions were assessed by radiologist, which were defined to 4 levels, 1. Resolution more than 50% (scores: 3 points), 2. Resolution between 10% and 50% (scores: 2 points), 3. Resolution less than 10% (scores: 1 point), 4. Pulmonary lesions progressed (scores: −3 points). The patients in the hydrogen/oxygen group showing higher scores at the 7th day, demonstrating that more patients had resolution of pulmonary lesions (Fig. 4, $p = 0.711$).

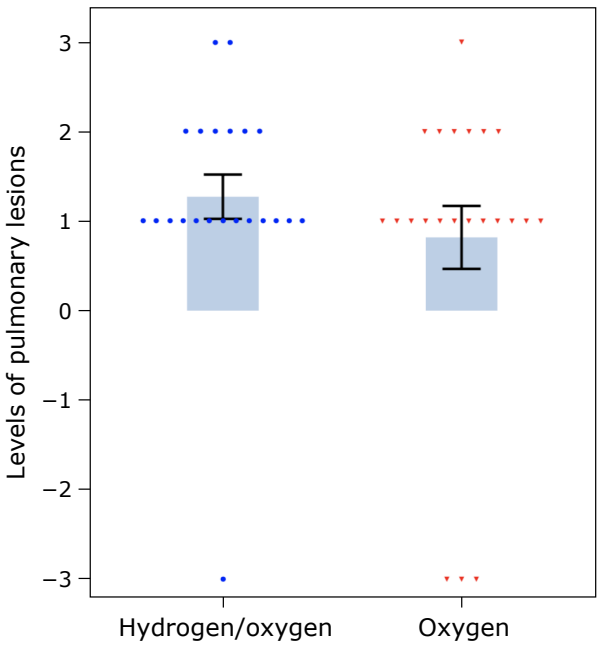

Fig. 4. The changes of chest CT images. Resolution more than 50% (scores: 3 points), between 10% and 50% (scores: 2 points), less than 10% (scores: 1 point), pulmonary lesions progressed (scores: −3 points).

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Adverse events. Only one patient was observed fever after hydrogen/oxygen mixed gas inhalation, and the investigator considered non-relationship with the Hydrogen/Oxygen Generators. No obvious and serious adverse events were found during the hydrogen/oxygen mixed gas inhalation process.

Discussion

This study is a multicenter, randomized, controlled trial to evaluate the efficacy of hydrogen/oxygen mixed gas inhalation as the adjuvant therapy for COVID-19 patients with variant Omicron. The present findings showed that hydrogen/oxygen inhalation shortened the virus shedding duration, decreased the inflammatory factor levels, as well as resolved the pulmonary lesions compared to oxygen inhalation alone, thereby reducing the occurrence of severe cases in the early stage.

Since the announcement that a mutation-laden coronavirus variant had been discovered in southern Africa, dozens of countries around the world have reported Omicron cases, including a worrying number of infections in people who have either been vaccinated or experienced previous SARS-CoV-2 infections.[8] But studies found that fast-spreading Omicron variant of the coronavirus SARS-CoV-2 was less dangerous than its predecessor Delta. Omicron replicates more readily in the upper airways than in the lung,[9] which was different from Delta.[10,11] Studies about the epidemiological characterization of the Omicron variant cases in Denmark and Norway, November to December 2021 demonstrated that there were 1.2% of the cases with Omicron variant hospitalized, while 1.5% with Delta variant. Besides, 0.13% cases with Omicron variant and 0.11% with Delta variant were admitted to intensive care unit.[12,13] The Omicron variant is highly transmissible and has extensive morbidity. Recent reports have revealed that the Omicron variant exhibits a longer cycle of viral shedding and a decreased replication capacity and results in substantially attenuated lung pathology, indicating that the pathogenic ability of the Omicron variant is lower than that of previous variants.[14] Worryingly, New subvariants of Omicron have emerged in succession, BA.2.12.1, BA.4, and BA.5 exhibiting higher transmissibility than others.[9] Cao *et al.*[15] suggested that three new variants had mutations that alter a key amino acid called L452. But like the earlier versions of Omicron, they have a remarkable ability to evade immunity from vaccines, previous infection, or both-a disturbing portent for the future of the pandemic and a potentially serious complication for vaccine developers. Xia *et al.*[16] identified that the T9I mutation in 2-E of the SARS-CoV-2, which may provide a possible explanation for the relatively mild pathogenicity of the Omicron variant. Some epidemiological studies showed that critical patients presented elevated levels of inflammatory factors, such as IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1a, and TNF-α in plasma as compared with mild cases. These drive the recruitment of immune cells such as macrophages, neutrophils, and T cells into the sites of infection, causing destabilization of endothelial cell to cell and the vascular barrier and diffusing alveolar damage, and ultimately leading to multi-organ failure and subsequent death.[4,11,17,18]

Hydrogen has been shown to have antioxidant, anti-inflammatory, hormone-regulating, and apoptosis-resistance properties. Based on a review of the research, the use of hydrogen might reduce the destructive cytokine storm and lung injury caused by SARS-CoV-2 during COVID-19 in the early stage, stimulating ropy sputum drainage, and ultimately reducing the incidence of severe disease.[5,19,20] Many reports described possible mechanisms of molecular hydrogen actions against different diseases.[5,21–24] Several articles had also been published about the potential benefits of molecular hydrogen therapy for COVID-19.[25] Meanwhile Yin *et al.*[26] aimed to investigate the protective role of molecular hydrogen in LPS-induced lung injury and demonstrated that inhalation of molecular hydrogen could relieve LPS-induced acute lung injury through down-regulating the TLR4-mediated NF-κB signaling pathway.

Our study showed the beneficial trends of molecular hydrogen in treating patients with COVID-19, nevertheless, limitations should be mentioned. First, some patients had been confirmed PCR-positive for several days at the enrolling time, which affected the precise record of the virus shedding duration. The relatively small sample might bring bios of the results, e.g., the anti-inflammatory trend was seen after hydrogen/oxygen inhalation, but without any statistical significance. Second, the operating time of hydrogen/oxygen inhalation was relatively short, for some patients had got the negative conversion of SARS-CoV-2 in the first three days.

In conclusion, due to the effect of molecular hydrogen on tackling both hypoxia and oxidative stress, as well as the results of our study, inhalation of molecular hydrogen may offer a prospective solution to adjuvant therapy for COVID-19 Patients.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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