


Hydrogen-oxygen therapy alleviates clinical symptoms in twelve patients hospitalized with COVID-19

A retrospective study of medical records

Peng Luo, MS^a, Yuanfang Ding, MS^b, Yuan He, PhD^c, Dafeng Chen, PhD^d, Qing He, MS^c, Zufeng Huang, MS^{a,b}, Shian Huang^{a,*}, Wei Lei, PhD^{a,c,d} 

Abstract

A global public health crisis caused by the 2019 novel coronavirus disease (COVID-19) leads to considerable morbidity and mortality, which bring great challenge to respiratory medicine. Hydrogen-oxygen therapy contributes to treat severe respiratory diseases and improve lung functions, yet there is no information to support the clinical use of this therapy in the COVID-19 pneumonia.

A retrospective study of medical records was carried out in Shishou Hospital of Traditional Chinese Medicine in Hubei, China. COVID-19 patients (aged ≥ 30 years) admitted to the hospital from January 29 to March 20, 2020 were subjected to control group (n=12) who received routine therapy and case group (n=12) who received additional hydrogen-oxygen therapy. The clinical characteristics of COVID-19 patients were analyzed. The physiological and biochemical indexes, including immune inflammation indicators, electrolytes, myocardial enzyme profile, and functions of liver and kidney, were examined and investigated before and after hydrogen-oxygen therapy.

The results showed significant decreases in the neutrophil percentage and the concentration and abnormal proportion of C-reactive protein in COVID-19 patients received additional hydrogen-oxygen therapy.

This novel therapeutic may alleviate clinical symptoms of COVID-19 patients by suppressing inflammation responses.

Abbreviations: Alb = albumin, ALT = aspartate aminotransferase, AST = alanine aminotransferase, CK = creatine kinase, CK-MB = CK isoenzymes, COVID-19 = SARS-CoV-2 caused acute pneumonia disease, CRP = C-reactive protein, CysC = cystatin C, DBil = direct bilirubin, GGT = glutamyl transferase, Glo = globulin, LDH = lactate dehydrogenase, ROS = reactive oxygen species, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, TP = total protein, Ure = urea.

Keywords: COVID-19, hydrogen-oxygen therapy, infection, retrospective, SARS-CoV-2

1. Introduction

In December 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused acute pneumonia disease (COVID-19) broke out in Wuhan, China, and quickly spread to many countries worldwide, especially in Europe, America, and South-East Asia.^[1-4] By May 1, 2021 globally, over 153 million cases and more than 3.2 million deaths

with COVID-19 disease have been confirmed globally from World Health Organization (WHO) situation report.^[4] Compared with SARS, which also spread around the world in 2003, SARS-CoV-2 was more contagious. Compared with flu, SARS-CoV-2 led to a higher mortality rate.^[5,6] On January 30, 2020, the WHO declared the COVID-19 pneumonia as a “Public Health Emergency of International Concern”, and on March 12,

Editor: Pavan Kumar.

PL, YD, and YH contributed equally to this work.

This paper was supported by the Novel Coronavirus Infection Control Technology and Product Program in Zhanjiang (2020E01009), Novel Coronavirus Pneumonia Prevention and Control Research Program in Guangdong Education Department (2020KZDZX1107).

The project and study were approved by the ethical committee (No PJ2020-029).

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

^a Cardiovascular Medicine Center, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong, China, ^b Cardiology Department, Shishou Hospital of Traditional Chinese Medicine, Jingzhou, Hubei, China, ^c Laboratory of Cardiovascular Diseases, Guangdong Medical University, Zhanjiang, Guangdong, China, ^d Department of Precision Laboratory Medicine, Affiliated Hospital of Guangdong Medical University, Zhanjiang, Guangdong, China.

* Correspondence: Shian Huang, Affiliated Hospital of Guangdong Medical University, 57 Renmin Southern Road, Xiashan, Zhanjiang, Guangdong 524001, China (e-mail: huangshian1969@126.com)

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Luo P, Ding Y, He Y, Chen D, He Q, Huang Z, Huang S, Lei W. Hydrogen-oxygen therapy alleviates clinical symptoms in twelve patients hospitalized with COVID-19: a retrospective study of medical records. *Medicine* 2022;101:9(e27759).

Received: 15 February 2021 / Received in final form: 8 August 2021 / Accepted: 28 October 2021

<http://dx.doi.org/10.1097/MD.00000000000027759>

it officially declared COVID-19 a global pandemic, this means not only an escalation in the severity of the epidemic, but also an escalation in the difficulty of fighting it.^[17] Due to the lack of medicines and vaccines, basic health services for COVID-19 are often interrupted. The number of COVID-19 deaths increases with age especially over 80 years old; moreover, basal diseases such as cardiovascular, cerebrovascular, and respiratory diseases increase the risk of death for COVID-19 patients and lead great difficulties to treatment.^[14] Therefore, there is an urgent need for effective therapeutics to not only reduce the infection and fatality rates of COVID-19, but also improve the survival status of patients.

Although Remdesivir, Chloroquine, Hydroxychloroquine, etc, have proven to be effective for the treatment of COVID-19, clinical data indicates that these small molecule drug candidates exhibited multiple side effects, for instance, headache, dizziness, diarrhea, stomach cramps, vomiting, arrhythmia, low blood pressure, liver inflammation, and sweating.^[8–11] Hypoxia initiated by lung injury leads to multiple organ damage^[12–14]; thus, alleviating respiratory distress can reduce the risk of death in severe COVID-19 patients. Combined with anti-inflammatory and potential viricidal properties and relative high security, oxygen therapy has been reported to reverse the hypoxia caused by the SARS-CoV-2 virus.^[15] Recently, molecular hydrogen (H₂) has been applied as therapeutic medical gas to reduce various organ system dysfunctions and improve survival rate due to its anti-oxidant, anti-inflammatory, and anti-apoptotic actions. It has been found that inhaled hydrogen therapy ameliorated acute lung injury in animal models^[16–18]; hence, we proposed a hypothesis that hydrogen-oxygen therapy may be beneficial to COVID-19 patients in protecting organs from hypoxia-induced damage.

Hydrogen-oxygen nebulizer therapy, an interesting new strategy that has been officially recommended by the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China,^[19] is of great significance to verify its validity. A multicenter, randomized, double-blind, parallel-group controlled trial has shown that hydrogen-oxygen therapy is superior to oxygen therapy in terms of safety and tolerability in patients with acute exacerbation of chronic obstructive pulmonary disease.^[20] Oxygen therapy and noninvasive mechanical ventilation have limited effect on the emergency treatment of tracheal stenosis,^[21] and also have important application in the treatment of critical respiratory failure in intensive care unit. Low density and low molecular weight gaseous helium (He) has been shown to reduce airway resistance and inspiratory force in patients with upper airway obstruction.^[22,23] However, due to the high cost of helium storage and production, it cannot be widely used in clinic. So far, in the emergency treatment of tracheal stenosis, there is no other way to reduce the inspiratory capacity satisfactorily except He inhalation. H₂ has similar physical properties to He, so it may reduce the airway resistance of narrow trachea. In addition, hydrogen can be produced easily and cheaply by electrolyzing water, it can even be available in ambulance.^[24] Hydrogen generator is new equipment, which has been reported to be used in the deposition of calcium oxalate in kidney, cerebral ischemia-reperfusion injury and chronic obstructive pulmonary disease model rats, and it showed good effects in reducing inflammation and biological safety.^[25–27] However, up to now, the clinical application of hydrogen-oxygen therapy in COVID-19 patients has not been reported.

In this study, we retrospectively collected and analyzed the detailed clinical data of SARS-CoV-2-infected patients diagnosed in the laboratory of Shishou Hospital of Traditional Chinese Medicine (Jingzhou, Hubei). In this study, we analyzed the clinical characteristics of COVID-19 pneumonia patients, evaluated the effectiveness and safety of hydrogen-oxygen mixture generated by medical hydrogen-oxygen nebulizers in the treatment of COVID-19, in viewing to provide a new clinical therapy for the prevention and treatment of this major infectious disease.

2. Materials and methods

2.1. Study design and patients

We did a retrospective review of medical records from 24 adult patients with moderate and severe COVID-19 pneumonia admitted to Shishou Hospital of Traditional Chinese Medicine from January 29 to March 20, 2020. COVID-19 is diagnosed according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China.^[19] The definition of severe patients is that adult meets any of the following items: shortness of breath, respiratory rate > 30 times min⁻¹; resting state, oxygen saturation is less than 93%; arterial oxygen partial pressure /oxygen inhalation is less than 300 mm Hg (1 mm Hg=0.133 kPa); pulmonary imaging showed that the lesions progressed more than 50% in 24 to 48 hours.

The patients recruited in this study meet any of the following criteria: patients were diagnosed as COVID-19; patients were aged between 18 and 80 years; patients were treated with nebulized hydrogen-oxygen mixture. Exclusion criteria: patients who have withdrawn from hydrogen-oxygen therapy; patients accompanied with liver, kidney, and heart diseases.

This study was reviewed and approved by the Medical Ethical Committee of Affiliated Hospital of Guangdong Medical University (Ethical approval No. PJ2020-029). Written informed consent was obtained from each enrolled patient.

2.2. Data collection

Clinical records, laboratory findings, and chest computed tomography (CT) scans were reviewed for 24 patients hospitalized with COVID-19. All information is obtained and managed through custom data collection tables, and 2 researchers independently collected and reviewed data collection tables to verify the accuracy of the data.

Basic clinical information of new admitted patients was collected, including gender, age, smoking history, other disease histories. Also, whether patients have direct contact with other COVID-19 patients were investigated. The interval time of CT examination, clinical symptoms including fever, cough, expectoration, fatigue, sore throat, diarrhea, and the results of the first and last laboratory examinations including immune inflammation indicators, electrolytes, myocardial enzyme profile, liver function, and renal function as well as the clinical status, cured, discharged, in treatment, critical, and death up to the study date was analyzed.

2.3. CT examination method

Chest plain scan was performed with United imaging 40 row CT instrument. The scanning parameters were: thickness 2.00 mm, cols 512, rows 512, zoom 1.00, window level 600, window width

1600. The patient was in supine position. The scanning range was from the apex to the costophrenic angle at the bottom of the lung. Two senior radiographers in the cardiothoracic group reviewed the films, analyzed the distribution, scope, shape, pleural effusion, lymph node enlargement of the patients, and analyzed the imaging manifestations of different clinical types and the relationship with the course of disease. To facilitate the statistics, the left and right lungs were divided into 10 segments, a total of 20 segments.

2.4. Laboratory diagnosis

According to WHO guidelines and regulations of the Chinese National Health Commission, throat swab samples of COVID-19 patients were collected, and quantitative RT-PCR recommended by China Center for Disease Control and Prevention was used to detect SARS-CoV-2. All laboratory work was completed by staff from Da An Gene Co, Ltd. of Sun Yat-Sen University and COVID-19 positive case was certified according to the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China.^[19]

2.5. Hydrogen-oxygen therapy

All the recruited patients received antiviral and symptomatic treatment, and 12 of them were additionally inhaled with hydrogen-oxygen mixture produced by the Medical Hydrogen-Oxygen Generator with Nebulizer (AMS-H-03, Asclepius Meditec Co, Shanghai, China), which was listed by the State Food and Drug Administration in national innovation 3 categories of respiratory medical equipment. As shown in previous reports, the Medical Hydrogen-Oxygen Generator with Nebulizer that has been approved for public use can provide an output for the hydrogen-oxygen gas mixture comprising 33% O₂ and 66% H₂ of 2000 to 3000 mL per minute by electrolysis of pure water.^[28]

2.6. Statistical analysis

SPSS 20.0 was used for statistical analysis. Continuous variables are expressed as median and interquartile range, and classified variables are expressed as frequency and percentage (%). The difference of continuous variables before and after treatment was compared by Mann-Whitney *U* test, and the difference of classified variables was compared by chi square test and Fisher exact test.

Table 1
Analysis of clinical baseline indicators.

Characteristic	Index	Case	Control	P
Age, median (IQR)—yrs		51.5 (41.25–59)	54.5 (40.75–61.5)	.644
Age group—n (%)				
	<30 yrs	0 (0)	0 (0)	1.000
	30–39 yrs	2 (16.67)	2 (16.67)	
	40–49 yrs	3 (25)	3 (25)	
	≥50 yrs	7 (58.33)	7 (58.33)	
Gender—n (%)				
	Female	7 (58.33)	6 (50)	1.000
	Male	5 (41.67)	6 (50)	
Smoking—n (%)				
	Yes	1 (8.33)	0 (0)	1.000
	No	11 (91.67)	12 (100)	
Clinical typing—n (%)				
	Mild type	0 (0)	0 (0)	1.000
	Moderate type	9 (75)	10 (83.33)	
	Severe type	3 (25)	2 (16.67)	
	Critical type	0 (0)	0 (0)	
Symptoms—n (%)				
	Fever	10 (83.33)	10 (83.33)	1.000
	Chills	2 (16.67)	1 (8.33)	1.000
	Headache	0 (0)	3 (25)	.217
	Fatigue	11 (91.67)	10 (83.33)	1.000
	Shortness of breath	4 (33.33)	1 (8.33)	.317
	Sore throat	4 (33.33)	6 (50)	.680
	Cough	11 (91.67)	10 (83.33)	1.000
	Sputum production	5 (41.67)	3 (25)	.667
	Chest pain	1 (8.33)	0 (0)	1.000
	Diarrhoea	2 (16.67)	1 (8.33)	1.000
Signs of infection—n (%)				
	Throat congestion	10 (83.33)	6 (50)	.193
	Tonsil swelling	2 (16.67)	2 (16.67)	1.000
	Enlargement of lymph nodes	0 (0)	0 (0)	1.000
Abnormal Chest CT—n (%)		12 (100)	12 (100)	1.000
Treatment outcome—n (%)				
	Death	0 (0)	1 (8.33)	1.000
	Discharge	12 (100)	11 (91.67)	

3. Results

3.1. Analysis of clinical baseline indicators

All the patients had a COVID-19 contact history, which was consistent with the epidemiological characteristics. The patients were divided into 2 groups, as those who were subjected to receive additional hydrogen-oxygen therapy (case group, $n = 12$), and those who were not (control group, $n = 12$). The clinical baseline indicators are summarized in Table 1. The proportions of male/female in case group and control group are 5/7 and 6/6, and median ages were 51.5 (41.25–59) and 54.5 (40.75–61.5) years, respectively, which are not significantly different between the 2 groups. The clinical types are moderate and severe, which represent the main types of COVID-19. Fever, fatigue, cough, pharyngeal hemorrhage, and tonsil enlargement are the main clinical manifestations. All 24 patients underwent chest CT scan and showed typical imaging features, such as multiple ground glass shadows of the lung. All the patients in case group survived and discharged, while a patient in control group died during hospitalization. These findings indicate that SARS-CoV-2 mainly causes clinical manifestations of inflammation and respiratory infections.

3.2. Analysis of related immune inflammation indicators

In this study, we tested and compared the clinical indicators related to immune inflammation in COVID-19 patients, including white blood cell count, neutrophil count, lymphocyte count,

monocyte count, neutrophil percentage, lymphocyte percentage, monocyte percentage, and C-reactive protein (CRP) (Table 2). As shown in Table 2, although the neutrophil percentage and the concentration and abnormal proportion of CRP before therapy have no significant differences between case group and control group, they are significantly lower in case group than in control group after therapy. The results indicate that antiviral, support, and other symptomatic treatments may make limited contribution to reducing inflammation, whereas hydrogen-oxygen therapy can help to suppress inflammatory responses induced by SARS-CoV-2 infection, so as to alleviate the clinical symptoms.

3.3. Analysis of electrolyte-related indexes

In this study, we tested and compared the electrolyte-related indicators of the hospitalized COVID-19 patients, including potassium, sodium, chlorine, total calcium, ionic calcium, CO₂-binding capacity, phosphorus, and magnesium (Table 3). The results show that the levels of potassium, sodium, chlorine, phosphorus, total calcium, and CO₂-binding capacity of some patients in the 2 groups were abnormal before therapy, while both the routine therapy and hydrogen-oxygen therapy greatly improved electrolyte disturbances. Moreover, there are no significant differences in these indexes between case group and control group after therapy. These findings suggest that hydrogen-oxygen, antiviral, support, and symptomatic therapy

Table 2
Analysis of indicators related to immune inflammation.

Variable	Before therapy			After therapy		
	Case	Control	P	Case	Control	P
White blood cell count						
Median (IQR)— $\times 10^9/L$	5.27 (4.34–6.14)	4.36 (3.07–7.76)	.488	4.82 (4.19–6.08)	5.49 (4.25–7.03)	.544
$> 9.5 \times 10^9/L$ —n (%)	0 (0)	2 (16.67)	.037	0 (0)	0 (0)	
$< 3.5 \times 10^9/L$ —n (%)	0 (0)	3 (25)		0 (0)	0 (0)	
Neutrophil count						
Median (IQR)— $\times 10^9/L$	3.29 (2.55–4.16)	2.83 (1.63–6.42)	.419	2.55 (2.35–4.32)	3.65 (2.89–5.21)	.126
$> 6.3 \times 10^9/L$ —n (%)	0 (0)	3 (25)	.014	1 (8.33)	0 (0)	1.000
$< 1.8 \times 10^9/L$ —n (%)	0 (0)	3 (25)		0 (0)	0 (0)	
Lymphocyte count						
Median (IQR)— $\times 10^9/L$	1.22 (1.05–1.7)	0.98 (0.67–1.35)	.133	1.5 (1.14–1.73)	1.19 (0.99–1.51)	.184
$> 3.2 \times 10^9/L$ —n (%)	0 (0)	0 (0)	.414	0 (0)	0 (0)	
$< 1.1 \times 10^9/L$ —n (%)	4 (33.33)	7 (58.33)		3 (25)	4 (33.33)	
Monocyte count						
Median (IQR)— $\times 10^9/L$	0.45 (0.38–0.54)	0.31 (0.16–0.43)	.024	0.36 (0.28–0.48)	0.36 (0.28–0.44)	.643
$> 0.6 \times 10^9/L$ —n (%)	1 (8.33)	1 (8.33)	1.000	0 (0)	0 (0)	1.000
$< 0.1 \times 10^9/L$ —n (%)	0 (0)	1 (8.33)		0 (0)	1 (8.33)	
Neutrophil percentage						
Median (IQR)—%	63.25 (56.88–68.85)	66.2 (50.58–84.65)	.564	58.1 (52.9–69.75)	66.55 (60.83–76.85)	.030
$> 75\%$ —n (%)	2 (16.67)	4 (33.33)	.640	2 (16.67)	4 (33.33)	.640
$< 40\%$ —n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
Lymphocyte percentage						
Median (IQR)—%	24.65 (20.7–32.33)	25.4 (8.55–33.23)	.665	29.45 (20.03–35.95)	21.5 (15.78–29.63)	.112
$> 50\%$ —n (%)	0 (0)	1 (8.33)	.1931	0 (0)	0 (0)	.667
$< 20\%$ —n (%)	2 (16.67)	5 (41.67)		3 (25)	5 (41.67)	
Monocyte percentage						
Median (IQR)—%	8.5 (7.38–9.45)	6.35 (4.05–8.2)	.013	6.7 (5.88–8.3)	6.1 (5.43–7.18)	.370
$> 10\%$ —n (%)	2 (16.67)	1 (8.33)	.590	1 (8.33)	1 (8.33)	1.000
$< 3\%$ —n (%)	0 (0)	2 (16.67)		1 (8.33)	1 (8.33)	
CRP						
Median (IQR)—mg/L	7.4 (3.45–23.2)	18.15 (4.83–43.33)	.184	2.4 (1.25–6.15)	7.95 (5.05–23.98)	.022
> 4 mg/L—n (%)	9 (75)	10 (83.33)	1.000	3 (25)	10 (83.33)	.012

Table 3
Analysis of electrolyte-related indexes.

Variable	Before therapy			After therapy		
	Case	Control	P	Case	Control	P
Potassium						
Median (IQR)—mmol/L	4.09 (3.79–4.25)	3.66 (3.55–4.14)	.166	4.32 (4–4.47)	4.49 (4.25–4.72)	.166
> 5.5 mmol/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	
< 3.5 mmol/L—n (%)	2 (16.67)	2 (16.67)		0 (0)	0 (0)	
Sodium						
Median (IQR)—mmol/L	139.36 (138.9–140.48)	139.45 (136.68–140.65)	.419	139.2 (138.57–140.13)	138.72 (138.26–140.01)	.624
> 147 mmol/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	
< 135 mmol/L—n (%)	0 (0)	1 (8.33)		0 (0)	0 (0)	
Chlorine						
Median (IQR)—mmol/L	99.17 (94.46–101.19)	103.35 (98.05–105.43)	.018	101.73 (100.96–102.4)	100.54 (95.61–101.41)	.053
> 108 mmol/L—n (%)	0 (0)	0 (0)	.640	0 (0)	0 (0)	.093
< 96 mmol/L—n (%)	4 (33.33)	2 (16.67)		0 (0)	4 (33.33)	
Total calcium						
Median (IQR)—mmol/L	2.31 (2.25–2.36)	2.16 (2.08–2.23)	.004	2.38 (2.37–2.41)	2.37 (2.26–2.4)	.201
> 2.8 mmol/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
< 2 mmol/L—n (%)	0 (0)	1 (8.33)		0 (0)	1 (8.33)	
Ionic calcium						
Median (IQR)—mmol/L	1.15 (1.13–1.18)	1.08 (1.04–1.12)	.005	1.19 (1.17–1.2)	1.18 (1.13–1.21)	.502
> 1.4 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	1.000
< 1 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	1 (8.33)	
CO ₂ -binding capacity						
Median (IQR)—mmol/L	27.3 (26.65–30.33)	23.9 (22.35–25.68)	.002	25.2 (24.1–28.8)	27.15 (23.95–30.55)	.506
> 29 mmol/L—n (%)	3 (25)	4 (33.33)	1.000	2 (16.67)	4 (33.33)	.371
< 23 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	1 (8.33)	
Phosphorus						
Median (IQR)—mmol/L	1.05 (0.92–1.17)	1.02 (0.88–1.14)	.623	1.21 (1.15–1.3)	1.16 (0.98–1.26)	.260
> 1.6 mmol/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
< 0.8 mmol/L—n (%)	0 (0)	1 (8.33)		0 (0)	1 (8.33)	
Magnesium						
Median (IQR)—mmol/L	0.99 (0.96–1.02)	0.96 (0.91–1.02)	.401	0.99 (0.92–1.04)	0.98 (0.89–1.08)	.795
> 1.25 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
< 0.65 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	

may maintain the electrolyte balance and improve respiratory acidosis to a certain extent.

3.4. Analysis of myocardial enzymes

In this study, we analyzed the related indexes of myocardial enzymes, such as lactate dehydrogenase (LDH), creatine kinase (CK), and CK isoenzymes (CK-MB) in 24 hospitalized

COVID-19 patients (Table 4). Before therapy, abnormal changes of LDH, CK, and CK-MB levels were observed in the 2 groups. Except the LDH levels of 2 patients in control group, all the indexes of myocardial enzymes returned to normal range after therapy; however, there are no significant differences in the changes of LDH between case group and control group. The results imply that hydrogen-oxygen therapy may have certain protective effect on the heart of COVID-19 patients.

Table 4
Analysis of myocardial enzyme profile.

Variable	Case	Control	P	After therapy		
				Case	Control	P
LDH						
Median (IQR)—U/L	220.15 (167.88–248.08)	149.45 (121.08–325.2)	.094	183.7 (135.08–196.38)	172.9 (141.45–227.68)	.707
> 240 U/L—n (%)	3 (25)	3 (25)	1.000	0 (0)	2 (16.67)	.331
< 100 U/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
CK						
Median (IQR)—U/L	48.85 (24.45–108.8)	53.8 (44.02–153.05)	.273	42.1 (26.53–57.6)	44.4 (40.78–65.73)	.544
> 194 U/L—n (%)	0 (0)	2 (16.67)	.331	0 (0)	0 (0)	
< 24 U/L—n (%)	3 (25)	1 (8.33)		0 (0)	0 (0)	
CK-MB						
Median (IQR)—U/L	8.2 (6.53–9.9)	10.2 (8.88–16.2)	.073	9.15 (7.7–10.65)	7.5 (5.23–12.28)	.452
> 25 U/L—n (%)	0 (0)	1 (8.33)	1.000	0 (0)	0 (0)	

3.5. Analysis of liver function-related indexes

In this study, we analyzed the liver function-related indexes of the hospitalized COVID-19 patients, such as serum direct bilirubin (DBil), indirect bilirubin (IBil), aspartate aminotransferase (ALT), alanine aminotransferase (AST), AST/ALT, Glutamyl Transferase (GGT), alkaline phosphatase (ALP), total protein (TP), Albumin (Alb), Globulin (Glo), Albumin/globulin, and total bile acid (Table 5). Before therapy, the levels of DBil, ALT, AST, GGT, ALP, TP, Alb, Glo, and Albumin/globulin exhibited abnormal changes in the COVID-19 patients. Although the changes of liver function indexes are not significant between case group and control group after therapy, the control group seemed to be worse than those before therapy. These results suggest that hydrogen-oxygen therapy has no hepatotoxic effect on patients with COVID-19.

3.6. Analysis of renal function-related indexes

In this study, we compared and analyzed the renal function related indexes of patients with COVID-19, including urea (Ure), creatinine, uric acid, and cystatin C (CysC) (Table 6). The results showed that the levels of Ure and CysC were abnormal in some patients of 2 groups before therapy; however, there are no significant differences in all the indexes between case group and control group after therapy, indicating that the renal function of patients with COVID-19 would not be affected by hydrogen-oxygen therapy.

4. Discussion

COVID-19 has become a global pandemic infectious disease. Due to the lack of specific antiviral drugs, supportive therapy is used

Table 5
Analysis of indexes related to liver function.

Variable	Before therapy			After therapy		
	Case	Control	P	Case	Control	P
DBil						
Median (IQR)— $\mu\text{mol/L}$	3.85 (2.83–6.28)	3.35 (2.58–4.28)	.470	3.3 (2.55–4.7)	3.6 (2.8–5.45)	.506
> 6.8 $\mu\text{mol/L}$ —n (%)	2 (16.67)	1 (8.33)	1.000	0 (0)	2 (16.67)	.478
IBil						
Median (IQR)— $\mu\text{mol/L}$	6.75 (5.1–10.68)	4.75 (3.97–7.1)	.046	6.2 (4.18–9.15)	6.6 (6.13–8.78)	.583
> 20 $\mu\text{mol/L}$ —n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
ALT						
Median (IQR)—U/L	28.65 (19.4–36.33)	23.05 (20.4–36.45)	.603	22.4 (18–36.68)	32.7 (18–48.83)	.507
> 45 U/L—n (%)	1 (8.33)	1 (8.33)	1.000	2 (16.67)	3 (25)	1.000
AST						
Median (IQR)—U/L	24.15 (19.75–28.95)	31.15 (22.73–45.6)	.094	20.3 (17.23–27.13)	25.2 (18.85–36.33)	.141
> 45 U/L—n (%)	0 (0)	3 (25)	.217	0 (0)	1 (8.33)	1.000
AST/ALT						
Median (IQR)	0.88 (0.66–1.07)	1.5 (0.96–1.75)	.008	0.84 (0.56–1.13)	0.98 (0.66–1.21)	.665
> 2.5—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
GGT						
Median (IQR)—U/L	35.05 (24.28–61.78)	32.75 (15.73–65.78)	.564	41.05 (34.18–67.65)	49.35 (29.38–71.23)	.977
> 50 U/L—n (%)	4 (33.33)	3 (25)	1.000	4 (33.33)	6 (50)	.680
ALP						
Median (IQR)—U/L	61.65 (51.73–75.35)	56.7 (49.98–75)	.603	62.2 (52.78–71.75)	65.3 (45.05–76.85)	.707
> 128 U/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	1.000
< 40 U/L—n (%)	0 (0)	1 (8.33)		0 (0)	1 (8.33)	
Total protein						
Median (IQR)—g/L	65.95 (62.63–74)	65.7 (61.05–70.53)	.885	68.4 (63.5–70.48)	65.2 (59.8–68.78)	.214
> 85 g/L—n (%)	0 (0)	0 (0)	1.000	0 (0)	0 (0)	.217
< 60 g/L—n (%)	1 (8.33)	1 (8.33)		0 (0)	3 (25)	
Albumin						
Median (IQR)—g/L	40.45 (35.7–44.45)	41.25 (38.55–44.6)	.623	42.15 (40.03–44.33)	39.85 (33.83–40.78)	.060
> 53 g/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	.400
< 40 g/L—n (%)	6 (50)	5 (41.67)		3 (25)	6 (50)	
Globulin						
Median (IQR)—g/L	25.6 (23.5–27.78)	23.25 (21.33–31.13)	.225	25.55 (22.85–28.3)	25 (22.68–32.85)	.885
> 32 g/L—n (%)	2 (16.67)	2 (16.67)	1.000	2 (16.67)	3 (25)	1.000
< 20 g/L—n (%)	0 (0)	0 (0)		1 (8.33)	1 (8.33)	
Albumin/globulin						
Median (IQR)	1.6 (1.3–1.78)	1.75 (1.35–1.98)	.401	1.5 (1.43–1.9)	1.65 (1.2–1.7)	.621
> 2.5—n (%)	0 (0)	0 (0)		1 (8.33)	0 (0)	1.000
< 1—n (%)	1 (8.33)	0 (0)		0 (0)	1 (8.33)	
Total bile acid						
Median (IQR)— $\mu\text{mol/L}$	2.65 (1.63–3.6)	2.9 (1.47–4.03)	.862	2.45 (1.85–4.15)	2.1 (1.8–3.73)	.563
>12 $\mu\text{mol/L}$ —n (%)	0 (0)	0 (0)		0 (0)	0 (0)	

Table 6
Analysis of indexes related to renal function.

Variable	Before therapy			After therapy		
	Case	Control	P	Case	Control	P
Urea						
Median (IQR)—mmol/L	3.13 (1.98–4.19)	3.39 (2.47–4.1)	.371	3.7 (2.4–4.68)	4.06 (3.04–6.99)	.175
> 7.14 mmol/L—n (%)	0 (0)	1 (8.33)	1.000	0 (0)	3 (25)	.093
< 1.43 mmol/L—n (%)	0 (0)	0 (0)		0 (0)	1 (8.33)	
Creatinine						
Median (IQR)— μ mol/L	62.1 (47.45–73.25)	68.9 (61.38–89)	.106	72.5 (60.68–78.53)	62.7 (56.9–76.08)	.402
> 97.2 μ mol/L—n (%)	0 (0)	0 (0)		1 (8.33)	0 (0)	1.000
< 35.4 μ mol/L—n (%)	0 (0)	0 (0)		0 (0)	0 (0)	
Uric acid						
Median (IQR)— μ mol/L	285.8 (244.83–334.25)	232.45 (218.53–310.63)	.356	271.1 (235.9–366.18)	302.05 (198.13–338.48)	.840
> 430 μ mol/L—n (%)	0 (0)	0 (0)		0 (0)	1 (8.33)	1.000
Cystatin C						
Median (IQR)—mg/L	0.62 (0.54–0.67)	0.62 (0.6–0.77)	.311	0.65 (0.58–0.72)	0.65 (0.6–0.8)	.707
> 1.05 mg/L—n (%)	0 (0)	0 (0)	.590	0 (0)	0 (0)	1.000
< 0.55 mg/L—n (%)	3 (25)	1 (8.33)		1 (8.33)	1 (8.33)	

as the main treatment method.^[29] Clinical studies have shown that the death of COVID-19 patients is characterized by typical pathological changes of lung parenchyma, and eventually develop progressive hypoxemia, lactatemia, acute respiratory distress syndrome, and respiratory failure.^[30–33] Hyperbaric oxygen therapy and normobaric oxygen therapy such as nasal duct and mask oxygen inhalation, noninvasive and invasive ventilation, and extracorporeal membrane oxygen have been widely used in the treatment of COVID-19; nonetheless, the mortality of severe patients remains high,^[31,32] and even more than 60%.^[31] This suggests that inhalation of excessive oxygen (O₂) may contribute to the detriment of survival in COVID-19 patients. Therefore, it is urgent to explore approaches for elimination of oxygen toxicity.

Hyperoxia, also called excess oxygen, increases formation of reactive oxygen species (ROS), and hence causes multiple-organ toxicity (e.g., central nervous, pulmonary, cardiac, and ocular toxicity).^[34] In contrast to O₂, H₂ is reductive, thereby it can selectively scavenge ROS (highly reactive hydroxyl radical, but not super oxide, peroxide, or nitric oxide) and has no serious side effects.^[35] Thus, we assumed that inhaled O₂ added with a certain concentration of H₂ may effectively protect organ functions against hyperoxia-induced toxicity. Hydrogen-oxygen nebulizers can provide hydrogen-oxygen mixed gas diffusing into the human body, reduce the flow resistance of gas in the bronchial tree, improve the utilization rate of H₂ and O₂, so as to provide auxiliary treatment for dyspnea and hypoxemia. In this study, we retrospectively observed the curative effect of hydrogen-oxygen therapy in 12 patients with moderate and severe COVID-19. The results of statistical analysis show marked improvement in immune-inflammation after treatment with hydrogen-oxygen mixture. More importantly, all the patients recovered and finally discharged. These findings suggest that hydrogen-oxygen therapy is conducive to alleviate inflammation, ameliorate dyspnea, and promote survival.

Recent studies suggest that oxidative stress is associated with RNA virus infection and pathogenesis including viral replication, immune response, apoptosis, inflammation, and organ dysfunction; thereby, it can enhance the severity of COVID-19.^[36–38] It has been demonstrated that SARS-CoV-2 can substantially promote mononuclear phagocyte system and neutrophil granulocytes to produce ROS which increase the formation of

neutrophil extracellular traps, suppress the adaptive immune responses, and cause organ damage.^[39,40] The systemic hypoxia induced by persistent hypoxemia should be treated with extra oxygen supply. However, oxygen therapy that may cause oxidative stress often facilitates COVID-19 patients to develop respiratory distress.^[41,42] We herein observed significant decrease of the neutrophil percentage and the concentration and abnormal proportion of CRP in COVID-19 patients who had received additional hydrogen-oxygen therapy (Table 2), suggesting that anti-oxidation, anti-virus, and anti-inflammation are probably involved in the underlying mechanism of this treatment method.

In the present study, we observed the abnormal elevations of LDH, CK, CK-MB, DBil, ALT, AST, GGT, Glo, and Ure and the decreases of TP, Alb, and CysC in a part of the patients before therapy by examining myocardial enzyme profile and functions of liver and kidney (Tables 4–6), suggesting that SARS-CoV-2 can damage heart, liver, and kidney. In addition, we found that the antiviral and symptomatic therapy seemed to further impair liver function (Table 5), indicating that the antiviral drugs may possess hepatotoxicity. Japanese scholars Katherine and Hayashida have demonstrated that breathing 2% H₂ can lighten liver and myocardial ischemia-reperfusion injury in animal models.^[43] However, we found that hydrogen-oxygen therapy suppressed the elevations of LDH and DBil and the decreases of TP and Alb with no statistical significance (Tables 4 and 5), which may be due to the small number of cases.

5. Conclusion

To our knowledge, this study is the first to demonstrate the curative effect of hydrogen-oxygen therapy on COVID-19; however, the sample size is the primary limitation. A prospective multicenter clinical trial is needed to clarify whether hydrogen-oxygen therapy is superior to hyperbaric oxygen therapy and normobaric oxygen therapy in COVID-19 treatment.

Acknowledgments

The authors thank the patients and staff from all the units who participated in the study, and thank Xiaorong Shui, Dafeng Chen, and Qing He for their contributions to this study.

Author contributions

PL and YD performed the experiments and collected the data. YH, DC, QH, and ZH organize and analyzed the data. WL and YH wrote the manuscript. SH and WL designed this study.

Data curation: Yuan He, Dafeng Chen, Qing He, Zufeng Huang.

Funding acquisition: Shian Huang.

Investigation: Peng Luo, Yuanfang Ding, Shian Huang, Wei Lei.

Methodology: Peng Luo, Yuanfang Ding, Yuan He.

Project administration: Wei Lei.

Resources: Peng Luo, Yuanfang Ding.

Validation: Shian Huang.

Writing – original draft: Wei Lei.

References

- [1] Franco C, Facciolo N, Tonelli R, et al. Feasibility and clinical impact of out-of-ICU non-invasive respiratory support in patients with COVID-19 related pneumonia. *Eur Respir J* 2020;3:2002130.
- [2] Tai DBG, Shah A, Doubeni CA, et al. The disproportionate impact of COVID-19 on racial and ethnic minorities in the United States. *Clin Infect Dis* 2020;20: ciaa815.
- [3] Salerno M, Sessa F, Piscopo A, et al. No autopsies on COVID-19 deaths: a missed opportunity and the lockdown of science. *J Clin Med* 2020;9:1472.
- [4] World Health Statistics 2021: visual summary. Retrieved June 2, 2021, Available at: <https://www.who.int/data/stories/world-health-statistics-2021-a-visual-summary>
- [5] Lai CC, Shih TP, Ko WC, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents* 2020; 55:105924.
- [6] Wang LS, Wang YR, Ye DW, et al. Review of the 2019 novel coronavirus (SARS-CoV-2) based on current evidence. *Int J Antimicrob Agents* 2020;55:105948.
- [7] WHO Director-General's opening remarks at the media briefing on COVID-19-11 March 2020
- [8] Chugh H, Awasthi A, Agarwal Y, et al. A comprehensive review on potential therapeutics interventions for COVID-19. *Eur J Pharmacol* 2021;890:173741.
- [9] Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020;395:1569–78.
- [10] Tang W, Cao Z, Han M, et al. Hydroxychloroquine in patients with mainly mild to moderate coronavirus disease 2019: open label, randomised controlled trial. *BMJ* 2020;369:m1849.
- [11] Nicol MR, Joshi A, Rizk ML, et al. Pharmacokinetics and pharmacological properties of Chloroquine and Hydroxychloroquine in the context of COVID-19 infection. *Clin Pharmacol Ther* 2020;108: 1135–49.
- [12] Wilkerson RG, Adler JD, Shah NG, et al. Silent hypoxia: a harbinger of clinical deterioration in patients with COVID-19. *Am J Emerg Med* 2020;38:2243e5–6.
- [13] Somers VK, Kara T, Xie J. Progressive hypoxia: a pivotal pathophysiological mechanism of COVID-19 pneumonia. *Mayo Clin Proc* 2020; 95:2339–42.
- [14] Xie J, Covassin N, Fan Z, et al. Association between hypoxemia and mortality in patients with COVID-19. *Mayo Clin Proc* 2020;95: 1138–47.
- [15] Jiang B, Wei H. Oxygen therapy strategies and techniques to treat hypoxia in COVID-19 patients. *Eur Rev Med Pharmacol Sci* 2020; 24:10239–46.
- [16] LeBaron TW, Kura B, Kalocayova B, et al. A new approach for the prevention and treatment of cardiovascular disorders. molecular hydrogen significantly reduces the effects of oxidative stress. *Molecules* 2019;24:2076.
- [17] Zheng Y, Zhu D. Molecular hydrogen therapy ameliorates organ damage induced by sepsis. *Oxid Med Cell Longev* 2016;2016:5806057.
- [18] Audi SH, Jacobs ER, Zhang X, et al. Protection by inhaled hydrogen therapy in a rat model of acute lung injury can be tracked in vivo using molecular imaging. *Shock* 2017;48:467–76.
- [19] National Health Commission of China: New coronavirus pneumonia prevention and control program (7th edn). March 4, 2020. Available at: <http://www.nhc.gov.cn/zycgj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml>
- [20] Zheng ZG, Sun WZ, Hu JY, et al. Hydrogen/oxygen therapy for the treatment of an acute exacerbation of chronic obstructive pulmonary disease: results of a multicenter, randomized, double-blind, parallel-group controlled trial. *Respir Res* 2021;22:149.
- [21] Skrinckas GJ, Hyland RH, Hutcheon MA. Using helium-oxygen mixtures in the management of acute upper airway obstruction. *Can Med Assoc J* 1983;128:555–8.
- [22] Jaber S, Carlucci A, Boussarsar M. Heliumoxygen in the postextubation period decreases inspiratory effort. *Am J Respir Crit Care Med* 2001; 164:633–7.
- [23] Rodeberg DA, Easter AJ, Washam MA, et al. Use of a helium-oxygen mixture in the treatment of postextubation stridor in pediatric patients with burns. *J Burn Care Rehabil* 1995;16:476–80.
- [24] Zhou ZQ, Zhong CH, Su ZQ, et al. Breathing hydrogen-oxygen mixture decreases inspiratory effort in patients with tracheal stenosis. *Respiration* 2019;97:42–51.
- [25] Peng Z, Chen W, Wang L, et al. Inhalation of hydrogen gas ameliorates glyoxylate-induced calcium oxalate deposition and renal oxidative stress in mice. *Int J Clin Exp Pathol* 2015;8:2680–9.
- [26] Cui J, Chen X, Zhai X, et al. Inhalation of water electrolysis-derived hydrogen ameliorates cerebral ischemia-reperfusion injury in rats—a possible new hydrogen resource for clinical use. *Neuroscience* 2016; 335:232–41.
- [27] Liu X, Ma C, Wang X, et al. Hydrogen coadministration slows the development of COPD-like lung disease in a cigarette smoke-induced rat model. *Int J Chron Obstruct Pulmon Dis* 2017;12:1309–24.
- [28] Wang R, Wu J, Chen Z, et al. Postconditioning with inhaled hydrogen promotes survival of retinal ganglion cells in a rat model of retinal ischemia/reperfusion injury. *Brain Res* 2016;1632:82–90.
- [29] World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected. [March 13, 2020]. Available at: <https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf>
- [30] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506.
- [31] Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020; 8:475–81.
- [32] Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020;323:1061–9.
- [33] Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020;395:507–13.
- [34] Thomson L, Paton J. Oxygen toxicity. *Paediatr Respir Rev* 2014;15:120–3.
- [35] Ohsawa I, Ishikawa M, Takahashi K, et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* 2007;13:688–94.
- [36] Reshi ML, Su YC, Hong JR. RNA viruses: ROS-mediated cell death. *Int J Cell Biol* 2014;2014:467452.
- [37] Suhail S, Zajac J, Fossum C, et al. Role of oxidative stress on SARS-CoV (SARS) and SARS-CoV-2 (COVID-19) infection: a review. *Protein J* 2020;39:644–56.
- [38] Chernyak BV, Popova EN, Prikhodko AS, et al. COVID-19 and oxidative stress. *Biochemistry (Moscow)* 2020;85:1543–53.
- [39] Schönrich G, Raftery MJ, Samstag Y. Devilishly radical NETWORK in COVID-19: Oxidative stress, neutrophil extracellular traps (NETs), and T cell suppression. *Adv Biol Regul* 2020;77:100741.
- [40] Borges L, Pithon-Curi TC, Curi R, et al. COVID-19 and neutrophils: the relationship between hyperinflammation and neutrophil extracellular traps. *Mediators Inflamm* 2020;2020:8829674.
- [41] Park HS, Kim SR, Lee YC. Impact of oxidative stress on lung diseases. *Respirology* 2009;14:27–38.
- [42] Mach WJ, Thimmesch AR, Pierce JT, et al. Consequences of hyperoxia and the toxicity of oxygen in the lung. *Nurs Res Pract* 2011;2011: 260482.
- [43] Hayashida K, Sano M, Ohsawa I, et al. Inhalation of hydrogen gas reduces infarct size in the rat model of myocardial ischemia-reperfusion injury. *Biochem Biophys Res Commun* 2008;373:30–5.