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RESEARCH ARTICLE

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Oral Liushen pill for patients with COVID-19: A prospective randomized controlled trial

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Abstract

We examined the efficacy and safety of Liushen pill combined with basic treatment for patients with COVID-19. In total, 181 patients hospitalized with COVID-19, classified as asymptomatic mild type, were randomly divided into the experimental ($n = 91$) and control ($n = 90$) groups and were administered placebo (Maizao decoction) and Maizao decoction and Liushen pill, in addition to standard care, respectively. The negative conversion rate of nucleic acid (Day 7), hospital discharge rate (Days 8, 10, and 14), symptom disappearance rate (Days 3, 5, and 7), inflammatory cytokine levels, and adverse events were compared between the groups. The negative viral conversion rate was significantly higher in the experimental than in the control group (48.35 vs. 31.11%, $p < 0.05$). Subgroup analysis showed a similar significant trend when the *Ct* value was ≤30 at baseline. After 10 days, the hospital discharge rate was significantly higher in the experimental than in the control group (69.23 vs. 53.33%, $p < 0.05$). After 3-day medication, the headache symptoms significantly disappeared in the experimental (88.57%) compared to the control group (63.33%) ($p < 0.05$). After 5 days, the symptom disappearance rates of headache and cough were significantly higher in the experimental (97.14%) than in the control group (97.14 vs. 80.00, $p < 0.05$; 82.65 vs. 58.93%, $p < 0.01$, respectively). Posttreatment, the procalcitonin level was significantly lower in the experimental than in the control group (0.09 ± 0.00 vs. 0.14 ± 0.05 ng/L; $p < 0.05$). There were no significant between-group differences in clinical safety test indices. Early intervention with Liushen pill improved cough and headache and increased negative viral conversion and discharge rate.

KEYWORDS

clinical trials, COVID-19 infection, Liushen pill, traditional Chinese medicine (TCM), virus negative conversion

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an acute respiratory human infectious disease caused by infection with the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is seriously harmful to human health.[1] Since the outbreak of the epidemic, SARS-CoV-2 has constantly mutated, resulting in several variants. The BA.2 subvariant of the Omicron SARS-CoV-2 strain has a higher infectivity, stronger vaccine breakthrough, and stronger antibody escape ability than prior variants. Therefore, it has spread to many countries and regions around the world to become the predominant epidemic strain in May 2022.[2,3] According to recent epidemiological statistics of the World Health Organization (WHO), as of June 5, 2022, there were >500 million confirmed COVID-19 cases worldwide, resulting in more than six million deaths.[4] The strong transmission and weak pathogenicity of the new variants have led to great changes in the concepts and strategies of global clinical diagnosis, treatment, and prevention management.[5]

According to the transmission characteristics and clinical manifestations of COVID-19, it is classified as a "Wenyi disease" in traditional Chinese medicine (TCM).[6,7] Since the outbreak of the pandemic in 2019, TCM has made an important contribution to the prevention and control of the epidemic and is one of the important treatment methods of COVID-19 infection.[8] Liushen pill is composed of musk, bezoar, toad, realgar, and six other medicinal herbs; however, the exact formulation is a national top-secret formula. Liushen pill allows the clearing away of heat and detoxification, while simultaneously exerting anti-inflammation effects and relieving pain. Liushen pill is a classic prescription for heat-clearing and detoxification of febrile diseases and has been used for >100 years. In recent years, a related research on Liushen pill has gradually deepened, and the pill has been found to exert various pharmacological activities, such as analgesia, anti-inflammation, cardiotonic effects, enhancing immunity, antitumor, and antivirus, and can further inhibit the expression of many kinds of novel coronavirus strains.[8,9] In clinical studies, Liushen pill has played a role in treating COVID-19 infection. A prospective randomized controlled study found that administration of Liushen pill can shorten the antipyretic time of patients with novel coronavirus infection and improve TCM syndromes and clinical symptoms.[10] The main functions of Liushen pill are heat-clearance and detoxification, anti-inflammation, and analgesia. It is recommended as a treatment for COVID-19 in many domestic regions of China for patients with fever, dry cough, sore throat, and headache. Considering the main respiratory infection of Omicron and the indication of Liushen pill, to further confirm the therapeutic effect of early intervention of Liushen pill on COVID-19, our hospital carried out a randomized controlled clinical trial of Liushen pill for the treatment of novel coronavirus infection (asymptomatic and mild type).

METHODS

Patient enrollment

This study adopted a prospective design and planned to include 200 patients with COVID-19 infection (asymptomatic, mild, and common type). Patients were randomly assigned to the experimental or control groups in a 1:1 ratio using the random table method.

Between April 10 and May 2, 2022, a total of 181 patients with asymptomatic infection and mild Omicron BA.2 were randomly assigned to the trial ($n = 91$) or control ($n = 90$) groups. All the patients completed their treatment. This study was registered in the China Clinical Trials Registration Center (ChiCTR2200058859).

Patients (1) with age ≥14 years; (2) who met the diagnostic criteria of the novel coronavirus infection based on the "novel coronavirus pneumonia diagnosis and treatment program (trial Ninth Edition)" issued by the National Health Commission; and (3) who voluntarily joined this study and signed the informed consent form, were included.

The exclusion criteria were as follows: (1) Presence of other respiratory tract infections caused by primary immunodeficiency disease, acquired immunodeficiency syndrome, congenital respiratory malformation, congenital heart disease, gastroesophageal reflux disease, pulmonary dysplasia and other basic diseases, or clear evidence of bacterial infection; (2) the following conditions: asthma requiring daily treatment, any other chronic respiratory diseases, respiratory bacterial infections such as suppurative tonsillitis, acute tracheobronchitis, sinusitis, otitis media, and other respiratory diseases, which may affect the evaluation of clinical trials; severe pulmonary interstitial lesions, bronchiectasis, and other basic lung diseases confirmed by chest CT; (3) severe pneumonia requiring mechanical ventilation; (4) a history of past or present illnesses, which may affect patients' participation in trials or affect the outcome of the study, including malignant diseases, autoimmune diseases, liver and kidney diseases, hematological diseases, nervous system diseases, and endocrine diseases; or diseases that seriously affect the immune (i.e., human immunodeficiency virus infection) or blood system, splenectomy, or organ transplantation;

(5) pregnant or lactating women; (6) participation in other clinical trials within 1 month; (7) allergies to two or more drugs or food or allergies to any of the ingredients of this drug; (8) cases of patients for whom the researchers believed that there were factors, which made them unsuitable for enrollment or could affect the evaluation of the efficacy of the Liushen pill.

Treatment plan

Patients in both groups were routinely managed in accordance with the requirements of the "novel coronavirus pneumonia diagnosis and treatment program (trial version 9)."[11] The experimental group was treated with the Liushen pill (batch number: UA01002, specification: 10 tablets/branch *six tablets/box; Lei Yun Shang Pharmaceutical Group Co., Ltd.). The participants received three sublingual pills and seven oral pills at a time, three times a day[12] and Maizao decoction (15 g fried malt, 10 g jujube, 200 ml decoction, one dose a day divided into two doses). The control group was treated with placebo (Maizao decoction). Each treatment course lasted 7 days; if the patient tested negative before this, the treatment was ended early.

Laboratory examination

Fasting venous blood was collected on hospitalization Day 1 or the morning of the next day to examine the lymphocyte and neutrophil (N) counts and the alanine transaminase (ALT), aspartate transaminase (AST), procalcitonin (PCT), interleukin-6 (IL-6), lactate dehydrogenase (LDH), D-dimer, serum creatinine (Cr), serum troponin C (c-TnT), and creatine kinase isoenzyme (CK-MB) levels. The reference range of each index is as follows: ALT: 9–52 U/L, AST: $1.10–3.20 \times 10^9$/L, PCT < 0.5 ng/L, IL-6: 3–5000 pg/L, lymphocyte count: 1.1–3.2 × 109/L, N: $1.8–6.3 \times 10^9$/L, LDH: 120–246 U/L, D-dimer: 0–550 μg/L, Cr: 46–92 μmol/L, c-TnT <14 pg/L, and CK-MB: < 3.61 ng/L.

Efficacy indicators

The main study objectives were: (1) the virus negative conversion rate of the two groups on Day 7 after admission; and (2) the discharge rate of the two groups on Days 8, 10, and 14 after admission. The secondary objectives were: (1) the effectiveness of clinical symptom improvement on Days 3, 5, and 7 after entering the group; and (2) the index of inflammatory factors: changes in the PCT, IL-6, and neutrophil count in the two groups before and on Day 7 after treatment.

Safety

The occurrence of any adverse events that occurred during the trial was recorded.

Discharge or release criteria

The discharge criteria were formulated according to the requirements of the "novel coronavirus pneumonia diagnosis and treatment program (trial version 9)"; viral negative conversion means that the *Ct* values of the ORF and *N* genes detected by the novel coronavirus were ≥35 on two consecutive measurements taken at least 24 h apart.

Statistical analysis

Data were processed using SPSS19.0 software (IBM Corp.). To describe the patients' baseline characteristics, we first statistically described the age, sex, BMI, history of basic diseases, and vaccination times of the randomly enrolled participants to ensure no differences in distribution and eliminate confounding factors. The secondary analysis was a comparison of the main and secondary study objectives between the treatment and control groups. The aforementioned statistical methods were applied according to the characteristics and distribution of the data. Especially, continuous variables were expressed as means ± standard deviations, and categorical variables were expressed as frequencies (n) and proportions (%). For continuous data, the difference between the groups, as observed using the *t*-test, Mann–Whitney *U* test, χ^2 test, and Fisher's exact test, was used to make rate comparisons. Additionally, the patients were divided into two subgroups: those with baseline *Ct* values ≤30 and >30. Then, according to the baseline *Ct* value, we compared the virus negative conversion rate of different baseline *Ct* values on Day 7 after enrollment.

RESULTS

Baseline data

A total of 181 patients with COVID-19 were randomly assigned to the control ($n = 90$) and experimental

($n = 91$) groups. The age, sex, body mass index (BMI), history of basic diseases, and vaccination times of the control (83 patients with asymptomatic infection and seven with mild infection) and experimental (89 patients with asymptomatic infection and three with mild infection) groups were compared (Table 1).

Negative conversion rate of the two groups on day seven after group allocation

On Day 7 after the group allocation, the virus negative conversion rate of the experimental group was 48.35% (44/91 participants), which was significantly higher than that of the control group (31.11%, 28/90 participants) ($p = 0.027$). According to the baseline *Ct* value, the patients were divided into two subgroups: those with baseline *Ct* value ≤30 and >30. The virus negative conversion rate was analyzed on Day 7. The results showed that when the baseline *Ct* value was ≤30, the virus negative conversion rates of the experimental and control groups on Day 7 were 44.26% and 25.40%, respectively ($p = 0.038$). When the baseline *Ct* value was >30, the virus negative conversion rate on Day 7 was 56.67% in the experimental group and 44.44% in the control group ($p = 0.437$) (Figure 1).

Discharge rates of the two groups on Days 8, 10, and 14 after admission

On Day 8 after admission, the discharge rates of the experimental and control groups were 23.08% (21/91 participants) and 16.67% (15/90 participants), respectively ($p = 0.371$). On Day 10 after the group allocation, the discharge rate of the experimental group was 69.23% (63/91 participants), while that of the control group was 53.33% (48/90 participants). The discharge rate was significantly higher in the experimental than in the

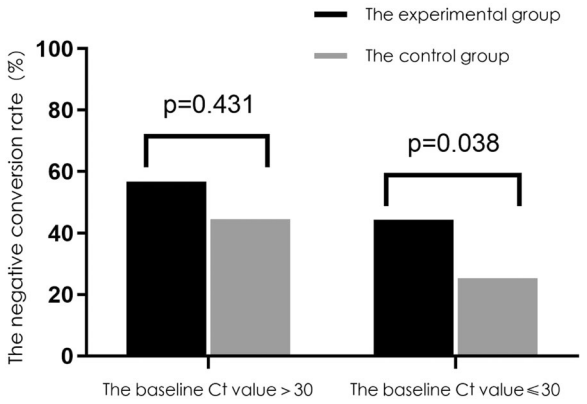

FIGURE 1 Comparison of virus negative conversion rate of different baseline *Ct* values on Day 7 after enrollment.

TABLE 1 Baseline data of the two groups.

	Group				
	Control group ($n = 90$)	**Experimental group ($n = 91$)**	**Total**	**Statistical value**	***p* value**
Age	40.06 ± 13.52	41.09 ± 14.69	40.57 ± 14.09	$t = -0.492$	0.623
Body mass index	23.67 ± 3.77	24.00 ± 4.03	23.84 ± 3.90	$t = -0.581$	0.562
Gender *n* (%)					
Male	41 (45.56)	39 (42.86)	80 (44.20)	$\chi^2 = 0.134$	0.715
Female	49 (54.44)	52 (57.14)	101 (55.80)		
Basic diseases history *n* (%)					
Yes	78 (86.67)	71 (78.02)	149 (82.32)	$\chi^2 = 2.323$	0.127
No	12 (13.33)	20 (21.98)	32 (17.68)		
Times of vaccination *n* (%)					
0	9 (10.00)	9 (9.89)	18 (9.94)	$\chi^2 = 3.131$	0.536
1	5 (5.56)	9 (9.89)	14 (7.73)		
2	48 (53.33)	40 (43.96)	88 (48.62)		
3	28 (31.11)	32 (35.16)	60 (33.15)		
4	0 (0.00)	1 (1.10)	1 (0.55)		

control group ($p = 0.041$). On Day 14 after the group allocation, the discharge rates of the experimental and control groups were 90.11% (82/91 participants) and 88.89% (80/90 participants), respectively ($p = 0.813$).

Disappearance rates of clinical symptoms on Days 3, 5, and 7 after group allocation

On Day 3, the disappearance rate of headache symptoms was significantly higher in the experimental (88.57%) than in the control group (63.33%) ($p = 0.020$). On Day 5, the disappearance rate of headache was still significantly higher in the experimental (97.14%) than in the control group ($p = 0.042$). Additionally, the disappearance rates of cough symptoms in the experimental and control groups were 82.76% and 58.93%, respectively. The disappearance rate was significantly higher in the experimental than in the control group ($p = 0.007$). On Days 3, 5, and 7, the disappearance rate of cough, pharyngeal discomfort, nausea and vomiting, fatigue, nasal congestion and runny nose, loose stools, body ache, and fever in the experimental group was higher than that in the control group, but there was no statistical significance between the two groups ($p > 0.05$, Tables 2–4).

Changes in inflammatory indexes before and after treatment

As shown in Table 5, there was no significant difference in the levels of PCT, IL-6, and lymphocyte and neutrophil counts between the two groups before the treatment. After the treatment, the level of PCT was significantly lower in the experimental than in the control group ($p < 0.05$).

Clinical safety monitoring index

During the study period, six participants in the experimental group experienced gastrointestinal adverse events, such as nausea and vomiting, and the incidence of adverse events was 6.59%. The researchers concluded that it might be related to research drugs, and after symptomatic treatment, all the patients returned to normal. No other drug-related adverse events were observed during the study, and no adverse events were observed in the control group. Other safety indicators during the study are presented in Table 6. However, there was no significant difference in safety indicators between the two groups before and after the treatment ($p > 0.05$).

DISCUSSION

The Omicron variant of SARS-CoV-2 was first discovered in 2021 and has since spread rapidly around the world, becoming the predominant strain. Most patients infected with Omicron have no or mild clinical symptoms,[13] but because of its high transmission rate, the global population base of infection is large, meaning that the number of related deaths remains high.[5] Additionally, the Omicron mutant has a higher infectivity and transmission concealment, allowing it to easily cause a large-scale epidemic outbreak in a short time, endanger people's health, and increase medical pressure. Therefore, the prevention and control of the Omicron mutant and the treatment of infected patients remain the focus of epidemic prevention.

COVID-19 is an epidemic disease caused by novel coronavirus, which belongs to fatal epidemic disease, called “Wen Yi” or “Wen Bing” in traditional Chinese medicine. According to the theory of TCM, “dampness and toxin” is the core pathological factor of COVID-19. There are many studies have proved that most of the TCM syndrome types of COVID-19 patients are “damp-toxin stagnant lung syndrome” and “epidemic toxin intercalation dryness syndrome,” so the principle of treatment is to “clearing heat and resolving dampness” which could help evil can be dispersed from “Weifen,” so as to prevent the disease from becoming worse[6,14] Liushen pill is a famous prescription for heat-clearing and detoxification of “Wen Bing,” and is a national secret variety. Therefore, it is only known that Liushen Pill is composed of six precious Chinese medicinal materials, such as musk, bezoar, realgar, and so on. Combined use of the above drugs can jointly promote blood circulation and remove blood stasis, clear heat and dampness, and diminish inflammation and relieve pain, especially headache, sore throat.[15]

To further confirm the efficacy and safety of the Liushen pill in the treatment of the novel coronavirus infection, in this clinical study, 181 patients with COVID-19 infection (asymptomatic, mild, and common type) were randomly divided into the experimental treatment ($n = 91$) and control ($n = 90$) groups. The results showed that on Day 7, the virus negative conversion rates of the experimental and control groups (both the ORF and *N* genes) were 48.35% and 31.11%, respectively. The negative conversion rate was significantly higher in the experimental than in the control group, and the difference was statistically significant ($p < 0.05$). At the same time, the results of subgroup analysis showed that when the baseline viral nucleic acid *Ct* value was <30, the virus negative conversion rate of the experimental group on Day 7 was significantly higher than that of the

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TABLE 2 Comparison of the disappearance rate of clinical symptoms on the 3rd day after entering the group.

	Experimental group			Control group			
Clinical symptoms	**Number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 3rd day (*n*)**	**Symptom disappearance rate on the 3rd day (%)**	**Number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 3rd day (*n*)**	**Symptom disappearance rate on the 3rd day (%)**	***p* value**
Pharynx discomfort	67	32	47.76	65	29	44.62	0.73
Pough	58	36	62.07	56	25	44.64	0.091
Nausea, vomiting or stomach discomfort	55	42	76.36	27	18	66.67	0.507
Fatigue	54	42	77.78	61	45	73.77	0.668
Asal congestion	54	37	68.52	54	32	59.26	0.423
Diarrhea in stool	51	33	60	33	16	48.48	0.213
Headache	35	31	88.57	30	19	63.33	0.020*
Muscle pain or soreness all over the body	31	27	87.1	31	26	83.87	1
Fever	23	22	95.65	31	28	90.32	0.628

Note: Compared with the control group, *$p < 0.05$.

TABLE 3 Comparison of the disappearance rate of clinical symptoms on the 5th day after entering the group.

	Experimental group			Control group			
Clinical symptoms	**Number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 5th day (*n*)**	**Symptom disappearance rate on the 5th day (%)**	**number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 5th day (*n*)**	**Symptom disappearance rate on the 5th day (%)**	***p* value**
Pharynx discomfort	67	49	73.13	65	42	64.62	0.348
Cough	58	48	82.76	56	33	58.93	0.007**
Nausea, vomiting or stomach discomfort	55	51	92.73	27	24	88.89	0.863
Fatigue	54	52	96.3	61	56	91.8	0.668
Asal congestion	54	45	83.33	54	42	77.78	0.628
Diarrhea in stool	51	41	74.55	33	24	72.73	0.578
Headache	35	34	97.14	30	24	80	0.042*
Muscle pain or soreness all over the body	31	30	96.77	31	26	87.1	0.351
Fever	23	23	100	31	30	96.77	1

Note: Compared with the control group, *$p < 0.05$, **$p < 0.01$.

TABLE 4 Comparison of the disappearance rate of clinical symptoms on the 7th day after entering the group.

	Experimental group			Control group			
Clinical symptoms	**Number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 7th day (*n*)**	**Symptom disappearance rate on the 7th day (%)**	**Number of occurrence cases (*n*)**	**The number of cases of disappearance of symptoms on the 7th day (*n*)**	**Symptom disappearance rate on the 7th day (%)**	***p* value**
Pharynx discomfort	67	56	83.58	65	50	76.92	0.458
Cough	58	50	86.21	56	42	75	0.202
Nausea, vomiting or stomach discomfort	55	55	100	27	26	96.3	0.718
Fatigue	54	54	100	61	58	95.08	0.288
Asal congestion	54	50	92.59	54	46	85.19	0.359
Diarrhea in stool	51	44	80	33	28	84.85	0.888
Headache	35	35	100	30	27	90	0.186
Muscle pain or soreness all over the body	31	31	100	31	28	90.32	0.237
Fever	23	23	100	31	31	100	1

TABLE 5 Comparison of immunological indexes before and after treatment.

		Experimental group	Control group	*p* value
Procalcitonin (ng/ml)	Before treatment	0.14 ± 0.03	0.12 ± 0.02	0.176
	After treatment	0.09 ± 0.00	0.14 ± 0.05	0.041*
Interleukin-6 (pg/ml)	Before treatment	6.67 ± 1.30	8.46 ± 1.33	0.051
	After treatment	3.90 ± 0.5857	4.55 ± 0.96	0.209
Neutrophil count (10^9/L)	Before treatment	4.61 ± 0.47	4.71 ± 0.38	0.738
	After treatment	3.66 ± 1.00	2.92 ± 0.38	0.162
Lymphocyte (10^9/L)	Before treatment	1.02 ± 0.53	0.90 ± 0.50	0.104
	After treatment	2.05 ± 0.55	2.11 ± 0.59	0.74

Note: Compared with the control group, *$p < 0.05$.

TABLE 6 Safety index of two groups of subjects before and after treatment.

		Experimental group	Control group	Statistical value	*p* value
Alanine transaminase	Before treatment	33.39 ± 29.06	30.57 ± 16.42	$t = 0.802$	0.424
	After treatment	40.06 ± 48.77	34.50 ± 28.51	$t = 0.495$	0.623
Aspartate transaminas	Before treatment	26.90 ± 13.82	24.21 ± 6.80	$t = 1.648$	0.102
	After treatment	25.53 ± 18.92	22.87 ± 10.02	$t = 0.633$	0.53
Lactate dehydrogenase	Before treatment	205.89 ± 36.42	203.31 ± 38.17	$t = 0.462$	0.645
	After treatment	189.00 ± 49.97	179.30 ± 39.64	$t = 0.733$	0.467
Serum creatinine	Before treatment	59.92 ± 15.63	57.77 ± 14.72	$t = 0.947$	0.345
	After treatment	57.69 ± 18.36	58.02 ± 13.15	$t = -0.070$	0.945
D-dimer	Before treatment	366.74 ± 341.86	374.00 ± 519.18	$t = -0.110$	0.912
	After treatment	351.25 ± 359.90	243.64 ± 121.18	$t = 0.930$	0.365
Serum troponin C	Before treatment	5.83 ± 3.75	5.75 ± 2.83	$t = 0.164$	0.87
	After treatment	9.13 ± 6.81	6.27 ± 2.00	$t = 1.149$	0.284
Creatine kinase isoenzyme	Before treatment	1.36 ± 0.92	1.25 ± 0.96	$t = 0.759$	0.449
	After treatment	0.93 ± 0.27	1.19 ± 0.83	$t = -0.827$	0.42

control group (44.26 vs. 25.40%, $p < 0.05$). Therefore, it was suggested that early treatment with Liushen pill can promote early negative transformation in patients with higher viral load. In vitro studies have confirmed that Liushen pill has a good inhibitory effect on the novel coronavirus, especially on the Omicron mutant.[16] However, this study confirmed for the first time that early intervention with the Liushen pill can promote the negative conversion of Omicron infection with fever, cough, sore throat, and headache, as the main symptoms. Considering the main characteristics of Omicron upper respiratory tract infection and that Liushen pill may cause gastrointestinal discomfort symptoms, in this study, Liushen pills were used for only 7 days. Moreover, fried malt, jujube medicine, and food homologous TCM were used to protect digestive function. The results showed that at 10 days after the group allocation, the discharge rate was significantly higher in the experimental (69.23%) than in the control group (53.33%) ($p < 0.05$). Additionally, the results showed that on Day 3, the clinical symptoms of patients in the experimental group showed a significant improvement trend, and the improvement rates of headache in the experimental and control groups were 88.57% and 63.33%, respectively, ($p < 0.05$). On Day 5, the improvement rate of headache in the experimental group (97.14%) was still significantly higher than that in the control group (80.00%) ($p < 0.05$). We hypothesize that this is related to the heat-clearing and detoxifying, anti-inflammatory, and analgesic effects of the Liushen pill; therefore, headache symptoms of the patients could be improved on Days 3 and 5 of treatment. Cough is the main clinical symptom of COVID-19 in the

acute phase and persists even after infection.[17] Cough also promotes transmission of respiratory droplets in communities, especially during the COVID-19 pandemic; thus, the control of COVID-19-related cough may reduce community transmission. This study found that on Day 5, the improvement rate of cough was significantly higher in the experimental than in the control group (82.65 vs. 58.93%, $p < 0.01$). To sum up, taking the Liushen pill can improve patients' clinical symptoms and increase patients' virus negative conversion and discharge rates, so as to save medical resources and reduce medical pressure.

The patients included in this study were all infected with the Omicron strain. Considering that Omicron has a low inhibition of interferon response in host cells, mainly invading the upper respiratory tract,[18] and that the proportion of viral pneumonia was also low, the level of inflammatory factors did not significantly increase before treatment. There was no significant difference in the levels of PCT and IL-6 between the experimental and control groups before treatment, but the levels of PCT and IL-6 were lower in the experimental than in the control group after treatment. Among them, the level of PCT in the experimental group was significantly lower than that in the control group (0.09 ± 0.00 vs. 0.14 ± 0.05 ng/L, $p < 0.05$). These results show that taking the Liushen pill can down-regulate the level of serum inflammatory factors and inhibit airway inflammation. In fact, the results of cytopathic effect in vitro (CPE) inhibition test showed that Liushen pill had a good inhibitory effect on SARS CoV-2, 501Y.V2/B.1.35, G/478K.V1/B.1.617.2. Transmission electron microscopy further confirmed that Liushen Pill could inhibit SARS CoV-2 from invading cells; The results of HE staining in vivo showed that Liushen Pill could significantly improve the acute lung injury induced by SARS CoV-2; In addition, RT-qPCR test in vitro and in vivo showed that Liushen pill could significantly inhibit the overexpression of inflammatory cytokines induced by SARS CoV-2 and 501Y.V2/B.1.35, and reduce the overexpression of inflammatory cytokines induced by G/478 K.V1/B.1.617.2 to varying degrees; Further in vitro and in vivo Western bolt tests showed that Liushen Pill could significantly reduce p-NF-κB p65、p-IκBα And p-p38 MAPK protein expression, and significantly upregulated IκBα Protein expression. It suggests that the molecular mechanism of Liushen Pill anti COVID-19 may be related to the regulation of NF-κB signal pathway by Liushen pill, thereby inhibiting the overexpression of inflammatory cytokines in the body.[9,19] The curative effect of the Liushen pill embodies the theoretical idea of "clearing heat and resolving dampness" in TCM for COVID-19 treatment. At present, many clinical and experimental studies have been performed to reveal the relevant molecular mechanism of "clearing heat and resolving dampness" in regulating airway inflammation and effectively relieving cough, fever, and other related clinical symptoms of patients with COVID-19.[14,20]

In conclusion, the Liushen pill combined with basic treatment can improve the condition of COVID-19 infection (asymptomatic, mild, and common type) and significantly increase the virus negative conversion and discharge rates of patients. This study further confirmed the efficacy and safety of Liushen pill in the treatment of COVID-19; these findings indicate it as a new medicine for the treatment of this disease. However, this study is only a single center clinical observation. Although randomized control and allocation concealment were adopted, there was no blind method and there was a certain risk of bias in the study. In the future, double-blind and multi-center large-sample studies should be carried out when conditions are ripe to further verify the research results.

AUTHOR CONTRIBUTIONS

Cuilin Shi, Jianan Huang, and Jianping Zhang conceived and contributed to the study design. Cuilin Shi and Jianping Zhang obtained ethical approval. Junheng Shen, Yian Liu, Wei Lei, and Xu Cao supervised the study. Zhong Yang and Junheng ShenTao Tao, Xu Cao, and Jing Lu participated in clinical treatment and data collection. Cuilin Shi and Junheng Shen were responsible for data management and analysis. Cuilin Shi drafted the manuscript. Cuilin Shi, Jianan Huang, and Jianping Zhang reviewed the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was reviewed and approved by the Ethics Review Committee of our hospital (approval No.: 2022-006) in April 2022. This study was conducted in accordance with the tenets of the Declaration of Helsink.

All written informed consent was obtained from all participants.

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