

Retrospective Analysis of the Four Seasons Antiviral Admixture in the Treatment of SARS-CoV-2 Infection in Children

Xixia Wang¹, Yile Kou¹, Qian Bai¹, Weihua Zhang³, Shasha Liu⁴, Hui Ding^{1,2,*}

¹Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China

²Department of Pediatric Intensive Care Medicine, The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, Xi'an 712046, Shaanxi, China

³Xianyang Children's Hospital (Xianyang Rainbow Hospital), Xianyang 712000, Shaanxi, China

⁴Shaanxi Haitian Pharmaceutical Company Limited, Xianyang 712000, Shaanxi, China

*Correspondence Author

Abstract: ***Objective:** To conduct a retrospective analysis, evaluate the clinical efficacy of the four seasons antiviral admixture, and provide clinical basis for the treatment of novel coronavirus infection in children. **Methods:** Using a clinical retrospective study method, A total of 102 children from the Second Affiliated Hospital of Shaanxi University of Chinese Medicine who met the diagnostic criteria of pediatric SARS-CoV-2 were collected, Randomized into control group (conventional western medicine treatment), 51 cases in the observation group (using four seasons antiviral admixture in the control group). Compare the changes of fever duration, the duration of cough, hospital stay, and before and after treatment (white oocyte count, percentage, percentage of lymphocytes, monocytes, C reactive protein, interleukin-6, sedimentation rate, creatine kinase isoenzyme, myoglobin, hypersensitive troponin I). **Results:** The cure rate was 76.47%, significantly higher than that of the control group ($P<0.05$); the difference between fever and duration of cough and hospitalization days in the observation and control groups ($P<0.05$); WBC count, central cell percentage, monocyte percentage, C reactive protein, interleukin-6, blood loss, increase of lymphocyte percentage and the difference ($P<0.05$); and no adverse events occurred after treatment. **Conclusion:** Four seasons antiviral admixture can significantly shorten the duration of fever, cough and hospitalization in children with novel coronavirus infection, effectively improve the infection index and myocardial injury index in children, and be safe and reliable.*

Keywords: The four seasons antiviral admixture, Children, SARS-CoV-2 infection, Virus toxicity bundle syndrome.

1. Introduction

The novel coronavirus (SARS-CoV-2) is a coronavirus of the genus β (Beta). After invasion of the human respiratory tract, it mainly relies on the receptor binding domain (RBD) on the S protein on its surface to recognize the host cell receptor angiotensin-converting enzyme 2 (ACE2) and bind to it to infect the host cell. The main clinical manifestations are dryness of the throat, sore throat, cough, fever, etc. Some patients may be accompanied by muscle aches and pains, decreased or loss of smell and taste, nasal congestion, runny nose, diarrhea, conjunctivitis and so on. In a few patients, the disease continues to develop with persistent fever and pneumonia-related manifestations [1]. As at July 23, 2023, More than 759 million confirmed cases and 6.8 million deaths have been reported in the global reporting pathway [2]. Reinfection occurs in approximately 0.65% of those infected with SARS-CoV-2 [3], and each infection experienced increases the risk of death, hospitalization, and pulmonary and extrapulmonary organ sequelae [4].

Frequent mutations in the genes of neocoronaviruses during epidemics and transmission in populations, As of the end of 2022, The World Health Organization (WHO) has proposed "variants of concern" (VOC) as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and Omicron (B.1.1.529). And the omicron mutant strain appeared in the population in November 2021, Compared to other VOC mutants such as Delta, The transmission and the immune escape of the Omicron has been significantly improved. and rapidly replacing the Delta variant as the absolute dominant

prevalent strain globally in early 2022 [1]. Researches have shown that omikron strains are more likely to invade the upper respiratory tract of children, presenting with hoarseness, barking cough, inspiratory laryngeal rales and dyspnea [5]. There are no approved antiviral drugs for new coronavirus infections in younger children of China, but TCM has been involved in the prevention and control of the epidemic in an all-encompassing and multidimensional manner, and has a unique role in reducing viral replication, anti-inflammation, immune regulation, protection of target organs, and improvement of clinical symptoms [6], and it has been included in the guidelines for the clinical treatment of new coronavirus infections.

According to Chinese medicine practitioners, the etiology of novel coronavirus infections is limited to the four ends of dampness, heat, toxicity and blood stasis which are categorized as "plague". Children's bodies are delicate, and the qi of yin and yang are more insufficient. Easily damaged by heat pathogens, the pestilent pathogens are heaty in nature, The heat pathogens move upwards and first attacks the lungs. The throat is the gateway to the lungs, heat pathogens attacks the lungs, causing the throat to be unfavorable, so it is easy to have hoarseness, cough, and sore throat, etc. Heat simmering within, Can't get through the skin, so it is likely to have a high fever that cannot be relieved. Treatment requires pungent cooling to relieve external pathogens and bitter cold to clear internal heat pathogens [7].

Four seasons antiviral admixture (State Drug Authorization: Z20027669) consists of 11 medicines: Fritillaria, Forsythia, Psidium guajava, Platycodon grandiflorum, Mulberry leaf,

Thornybush, Mentha piperita, Perilla leaf, Bitter almonds, Rehmannia, Chrysanthemum, Licorice. which used to Clearing away heat from the lungs and promoting the movement of pathogenic outward. Since December 2022, The Second Affiliated Hospital of Shaanxi University of Chinese Medicine has achieved good therapeutic effect on children admitted with new coronavirus infections by using four seasons antiviral admixture combined with the conventional treatment of Western medicine, and the case data are summarized and analyzed.

2. Objects and Methods

2.1 Objects

A total of 102 cases of children with novel coronavirus infection aged 2-12 years old, including 60 males and 52 females, hospitalized in the Second Affiliated Hospital of Shaanxi University of Chinese Medicine from December 2022 to April 2023 were selected. The study was reviewed and approved by the Ethics Committee of the Second Affiliated Hospital of Shaanxi University of Chinese Medicine.

2.2 Diagnostic Criteria

Refer to the Expert Consensus on the Diagnosis, Treatment and Prevention of Novel Coronavirus Infections in Children (Fifth Edition) [2] for diagnostic criteria of novel coronavirus infection: 1) with clinical manifestations related to SARS-CoV-2; 2) with 1 or more positive pathogenetic and serologic test results. The wind-cold pathogens attacks the surface of the body are characterized by the following clinical signs: fever and headache, no sweating, body aches and pains, itchy cough or dry and painful throat, sputum with little mucus, nasal congestion and turbid mucus. The tongue is red, the moss is thinly white or thinly yellow, and the pulse is floating [1].

2.3 Inclusion Criteria

- 1) Hospitalized children aged 2 to 12 years old;
- 2) Mildly hospitalized children who meet the above diagnostic criteria;
- 3) Oral administration of four seasons antiviral admixture during hospitalization;
- 4) The Chinese medicine evidence is in accordance with the wind-cold pathogens attacks the surface of the body.

2.4 Exclusionary Criteria

- 1) Children with severe and critical forms of novel coronavirus infection;
- 2) Children with other pathogenic infections in combination;
- 3) Children with other primary diseases of the lungs, or children with severe diseases of other systems of the body;

- 4) Children with various immunodeficiency diseases;
- 5) Children with incomplete medical records;
- 6) Children who did not meet the epidemiological evidence of wind-cold pathogens attacks the surface of the body;
- 7) Children taking other proprietary Chinese medicines except four seasons antiviral admixture during hospitalization.

2.5 Grouping and Medication

Hospitalized children were randomly assigned to a control group or an observation group if they met the diagnostic criteria for a new coronavirus infection.

The control group was treated routinely: Recombinant human interferon alpha 1b for injection, 1/4 tensor glucose saline rehydration solution for IV rehydration, and ibuprofen suspension. Usage Dosage: Interferon alpha1b (State Drug Authorization: S10960058) 2ug/(kg-dose), po: 4ml for 2-3 years old, 5ml for 4-6 years old, 8ml for 7-9 years old, 10ml for 10-12 years old. The Observational group was given conventional treatment combined with four seasons antiviral admixture (State Drug Authorization: Z20027669), The prescription consists of 11 herbs, including Fritillaria, Forsythia, Psidium guajava, Platycodon grandiflorum, Mulberry leaf, Thornybush, Mentha piperita, Perilla leaf, Bitter almonds, Rehmannia, Chrysanthemum, Licorice. which used to Clearing away heat from the lungs and promoting the movement of pathogenic outward. po: 1~2 years old 3mL/dose; 2~5 years old 5mL/dose, 5~7 years old 5~10mL/dose, 7~12 years old 10~15mL/dose; 3 times/day.

2.6 Observation Indicators

- 1) Days of fever and cough disappearance and days of hospitalization;
- 2) Changes in monocyte count, monocyte percentage, C-reactive protein, leukocytes, neutrophil percentage, lymphocyte percentage, interleukin-6, and erythrocyte sedimentation rate before and after treatment.

2.7 Statistical Methods

SPSS 26 software was used for statistical analysis. Measurement data obeyed normal distribution and were expressed as mean \pm standard deviation ($\pm s$), and independent samples t-test was used for comparison between the two groups, and paired samples t-test was used before and after treatment within the group. Count data were expressed as rate (%) using chi-square test or exact probability method. $p < 0.05$ indicates that the comparison between the two groups is statistically significant and the conditional hypothesis is accepted; $p \geq 0.05$ indicates that the comparison between the two groups is not statistically significant.

3. Result

3.1 Comparison of Baseline Data between the Two Groups of Patients

By comparing the age, gender, co-morbidities, temperature, respiration and weight of the children in both groups, there was no statistical significance ($P>0.05$), as shown in Table 1.

3.2 Evaluation of Clinical Efficacy

Both treatment regimens were effective for children with new

coronavirus infection, and the cure rate of the observation group was 76.47%, which was significantly higher than that of the control group ($P<0.05$), as shown in Table 2, indicating that conventional Western medicine treatment combined with four seasons antiviral admixture was superior to conventional Western medicine treatment.

Table 1: Comparison of baseline data of children in observation group and control group

projects	Observational group (51 cases)	Control group (51 cases)	T/ χ^2	P
Age (years)	6.84 \pm 5.47	6.68 \pm 5.38	0.748	0.630
Gender [cases (%)]	Male [32(62.7%)] female[19(37.2%)]	male[28(55%)] female[23(45%)]		
Compound Diseases (Cases)	Broncho-pneumonia (19) Acute bronchitis (17) Acute laryngotracheitis (15)	Broncho-pneumonia(17) Acute bronchitis (17) Acute laryngotracheitis (18)		
temperature ($^{\circ}$ C)	38.42 \pm 1.05	38.29 \pm 0.83	0.719	0.679
Respirations (breaths/minute)	32.96 \pm 5.26	33.08 \pm 7.64	0.753	0.662
Weight (kg)	9.09 \pm 1.44	9.45 \pm 0.96	1.204	0.110

Table 2: Comparison of clinical efficacy between observation group and control group (cases, %)

groups	Cases	curable	produce an effect	validity	null	efficient	recovery rate	Z	P
control subjects	51	30	15	6	0	100%	58.82%		
Observation Group	51	39	10	2	0	100%	76.47%	-2.315	0.021

3.3 Comparison of Fever, Cough Duration and Hospitalization Days between the Two Groups of Children

The difference in the duration of fever and cough and the number of days of hospitalization of the children in the observation group compared with the control group was statistically significant ($p<0.05$), as shown in Table 3. It suggests that four seasons antiviral admixture reduces the duration of fever and cough of the children and significantly shortens the number of days of hospitalization of the children.

Table 3: Comparison of fever, duration of cough and number of days of hospitalization between two groups of children

Project (days)	Observational group	Control group	t	P
fever	2.78 \pm 0.08*	3.81 \pm 0.28	2.664	0.011
cough	4.31 \pm 0.33 $^{\Delta}$	5.73 \pm 0.25	3.385	0.002
Days of hospitalization	5.52 \pm 0.17*	6.68 \pm 0.05	3.198	0.003

Note: * Δ # \times P<0.05, the observation group is statistically significant compared with the control group.

3.4 Comparison of Infection Indicators between the TWO GROUPS of Children before Admission

There was no statistically significant difference between the two groups of children in terms of leukocyte count, centrophil percentage, lymphocyte percentage, monocyte percentage, C-reactive protein, interleukin-6, and blood sedimentation ($P>0.05$), and in comparison after treatment, the observation group's leukocyte count, centrophil percentage, monocyte percentage, C-reactive protein, interleukin-6, and blood sedimentation decreased more significantly than that of the control group, and the lymphocyte percentage rose significantly compared with the control group, and the difference was statistically significant ($P<0.05$), suggesting that four seasons antiviral admixture combined with conventional Western medicine has a significant effect in improving inflammatory indexes. See Table 4 for details.

Table 4: Comparison of inflammatory indicators before and after treatment in two groups of children

Inflammation Indicators	Observational Group pre-treatment	control Group post-treatment	control Group pre-treatment	post-treatment
Leukocyte count ($\times 10^9/L$)	13.26 \pm 0.73 $^{\#}$	5.53 \pm 0.86*	12.99 \pm 0.20	7.17 \pm 0.42
Centrophil percentage (%)	89.22 \pm 1.25 $^{\#}$	53.61 \pm 1.49*	90.11 \pm 1.03	74.15 \pm 1.12
Lymphocyte percentage (%)	6.25 \pm 0.37 $^{\#}$	31.79 \pm 0.33*	6.14 \pm 0.33	18.92 \pm 0.54
Monocyte percentage (%)	14.76 \pm 0.08 $^{\#}$	3.67 \pm 0.08*	14.55 \pm 0.24	9.59 \pm 0.26
C-reactive protein (mg/L)	30.36 \pm 10.72 $^{\#}$	5.72 \pm 2.20*	28.58 \pm 12.16	4.91 \pm 2.18
Interleukin-6 (pg/mL)	38.27 \pm 7.83 $^{\#}$	0.75 \pm 0.03*	37.94 \pm 7.25	6.84 \pm 1.02
Hematologic sedimentation (mm/h)	78.82 \pm 9.46 $^{\#}$	11.02 \pm 7.47*	80.13 \pm 9.59	15.31 \pm 8.77

Note: # $P>0.05$, there is no statistical significance of each index in the comparison between the observation group and the control group before treatment; * $P<0.05$, there is no statistical significance of each index in the comparison between the observation group and the control group after treatment.

3.5 Comparison of Myocardial Injury in Two Groups of Children

There was no statistical difference in the comparison of creatine kinase isoenzyme, myoglobin, and ultrasensitive troponin I between the two groups of children before admission ($P>0.05$), and in the comparison after treatment, the observation group decreased more significantly than the control group, and the difference was statistically significant ($P<0.05$), suggesting that the four seasons antiviral admixture combined with the conventional western medical treatment has a significant effect in reducing creatine kinase isoenzyme, myoglobin, and ultrasensitive troponin I. See Table 5 for details.

Table 5: Comparison of myocardial injury in two groups of children

Inspection Indicators	Observational Group		control Group	
	pre-treatment	post-treatment	pre-treatment	post-treatment
Creatine kinase isozyme (ug/L)	7.08±1.43 [#]	1.64±1.13 [*]	7.15±1.29	4.86±1.17
Myoglobin (ug/L)	52.14±2.38 [#]	3.48±0.63 [*]	51.97±0.99	3.19±0.83
Ultra-sensitive troponin I (ug/L)	0.13±0.25 [#]	0.02±0.11 [*]	0.13±0.17	0.04±0.06

Note: # P > 0.05, there is no statistical significance of each index in the comparison between the observation group and the control group before treatment; * P < 0.05, there is no statistical significance of each index in the comparison between the observation group and the control group after treatment.

3.6 Security Assessments

There were no statistically significant changes in liver and renal function before and after treatment in the observation group (see Table 6 and Table 7), direct bilirubin, indirect bilirubin, alanine aminotransferase, glutamic oxalate aminotransferase, urea nitrogen, creatinine, and cystatin C levels were all within the reference values, and there were no adverse events in the course of this study. This indicates that four seasons antiviral admixture is safe and reliable.

Table 6: Comparison of liver function before and after treatment of children in observation group

	direct bilirubin(um ol/L)	indirect bilirubin(um ol/L)	gammaglutaminase (U/L)	glutamic transaminase (U/L)
pre-treatment	1.89±0.11	7.76±1.39	23.15±3.29	25.20±4.01
post-treatment	1.75±0.16 [#]	7.63±1.31 [#]	22.98±3.34 [#]	24.85±3.98 [#]

Note: # P>0.05, the comparison of liver function before and after treatment was not statistically significant.

Table 7: Comparison of renal function before and after treatment of children in observation group

	urea nitrogen (mmol/L)	Creatinine (umol/L)	Blastocystatin C (mg/L)
pre-treatment	8.32±1.95	47.27±13.53	0.931±0.194
post-treatment	2.66±0.28 [#]	42.58±12.57 [#]	0.646±0.122 [#]

Note: # P>0.05, the comparison of renal function before and after treatment was not statistically significant.

4. Discussion

The main clinical manifestations of children with novel coronavirus infection are fever and cough [8-9], and some patients may have nasal congestion, runny nose and dyspnea. Western medicine mainly focuses on symptomatic supportive therapy and broad-spectrum antiviral therapy, and there is no specific drug for novel coronavirus [10], and because of the influence of children's physiopathological characteristics, children have more limited choices of drugs in the treatment of disease.

The efficacy of Chinese medicine in regulating immunity and anti-virus is remarkable, and it is one of the important tools in the current treatment of novel coronavirus infections. Because most people infected with the new coronavirus have the same

symptoms and epidemiological characteristics, it is categorized under the category of "plague" in Chinese medicine. The cause of the disease is mainly due to the feeling of pestilent pathogens. Children have the physiological characteristics of "delicate internal organs, not yet full of energy and qi." and "the young yin has not yet grown and the lungs are delicate". And the pestilent pathogen is included in the heat pathogens. Therefore, it's easy for the pestilent pathogen to attack the lungs with the wind-heat pathogen. It is manifested as: fever, cough, and malaise. Treatment is based on the method of "dispersing wind pathogens and relieving the surface, clearing heat pathogens and removing hazardous substance". four seasons antiviral admixture is made from the classic formula of Sang Ju Drink and Yin Qiao San for treating external wind-heat, which contains Fritillaria, Mulberry leaf, forsythia and other traditional Chinese medicines, which together play the role of clearing heat and removing hazardous substance and promoting the lungs function to stop coughing.

The results of the study showed that the four-season antiviral combination combined with conventional Western medicine treatment could significantly shorten the duration of fever and cough and the number of days of hospitalization in children with neocoronavirus infection, and was significantly more effective than conventional Western medicine treatment alone in improving clinical symptoms. four seasons antiviral admixture significantly improved the levels of Leucocyte Count, Centrophil Percentage, Lymphocyte Percentage, Monocyte Percentage, C-Reactive Protein, Interleukin-6, and Blood Sedimentation in children with Neocoronavirus infection, suggesting that four seasons antiviral admixture has an anti-inflammatory effect. Its anti-inflammatory effect is hypothesized to be related to the active ingredient in the four seasons antiviral admixture. four seasons antiviral admixture contains quercetin, lignans, kaempferol, β -sitosterol, stigmasterol, naringenin, chrysin, isorhamnetin, carotenoids, scutellarin. Such components may control disease progression by modulating multiple targets, as quercetin may act on AKT 1, MAPK 1, MAPK 14, and IL-6, which interact with ACE2 to diastole bronchial smooth muscle and attenuate pulmonary vascular permeability [11-13]; Lignans have high affinity with the main protease of SARS-CoV-2 coronavirus, inhibit viral protease activity, and have potential inhibitory effect on SARS-CoV-2 [14-15], which can inhibit viral proliferation, uptake ,thereby protecting virus-infected cells. However, the mechanism of action of the four seasons antiviral admixture for the treatment of new coronavirus infections still needs to be further explored and studied.

This study showed that four seasons antiviral admixture could reduce the indexes related to myocardial injury in children with neocoronavirus infection, and the effect was significant compared with that of the control group (P<0.05).On the one hand, it may be related to the reduction of inflammatory reaction after the anti-virus of four seasons antiviral admixture; on the other hand, the research group hypothesized that four seasons antiviral admixture may have a protective effect on cardiomyocytes, which has yet to be systematically analyzed and investigated.

This retrospective clinical study demonstrated the clear efficacy of the four seasons antiviral admixture in

combination with conventional Western medicine in the treatment of children with neocoronavirus infections, but there are no clinical data on the use of the four seasons antiviral admixture on its own, which makes the four seasons antiviral admixture clinically limited in the treatment of children with neocoronavirus infections. Furthermore, the active ingredients of four seasons antiviral admixture are complex, and the mechanism of action against Neocoronavirus has not been elucidated, which will affect its clinical application. Therefore, clinical studies on the use of four seasons antiviral admixture against Neocoronavirus without combining with conventional Western medicine need to be conducted, and its active ingredients and mechanism of action still need to be further explored.

References

- [1] National Health Commission of the People's Republic of China. Diagnosis and treatment plan for COVID-19 (trial version 10) [J]. Chinese Journal of Clinical Infectious Diseases, 2022, 16(1):1-9.
- [2] Jian Rongmeng. Diagnosis, treatment and prevention of severe acute respiratory syndrome coronavirus 2 infection in children: experts' consensus statement (Fifth Edition) updated for the Omicron variant[J]. Chinese Journal of Applied Clinical Pediatrics, 2023, 1(38):20-30.
- [3] Hansen CH, Michlmayr D, Gubbels SM, et al. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study[J]. Lancet, 2021, 397(10280):1204-1212.
- [4] Mu Xuechun, Wang Linhan. Advances in the study of novel coronavirus reinfection[J]. National Medical Journal of China, 2022, 102(40):3229-3232.
- [5] Cui X, Zhao Z, Zhang T, et al. A systematic review and meta-analysis of children with coronavirus disease 2019 (COVID-19) [J]. J Med Virol, 2021, 93(2): 1057-1069.
- [6] Ren W, Liang P, Ma Y, et al. Research progress of traditional Chinese medicine against COVID-19[J]. Biomed Pharmacother, 2021, 137: 111310.
- [7] Cui Heqing. Characteristics of Epidemic Febrile Disease Differentiation and Treatment in YE Tianshi and Its Enlightenment to Prevention and Treatment of Novel Coronavirus Infection in Children[J]. Chinese Archives of Traditional Chinese Medicine, 2023. 41(02): 15-18.
- [8] Ma XF, Liu SS, Chen LZ, et al. The clinical characteristics of pediatric inpatients with SARS-CoV-2 infection: A meta-analysis and systematic review[J]. J Med Virol, 2021. 93(1): 234-240.
- [9] Zhang Yifei. Clinical features of severe acute respiratory syndrome coronavirus 2 Omicron variant infection in children: an analysis of 201 cases[J]. Chinese Journal of Contemporary Pediatrics, 2023, 25(1): 5-10.
- [10] Shan Sisi, Yang Ziqin, Zhang linqi. Research Progress on Antibody Therapies Against SARS-CoV-2 Received US FDA Emergency Use Authorization[J]. Chinese Journal of Medical Guide, 2022, 24(1): 9-15.
- [11] Shorobi FM, Nisa FY, Saha S, et al. Quercetin: A Functional Food-Flavonoid Incredibly Attenuates Emerging and Re-Emerging Viral Infections through Immunomodulatory Actions[J]. Molecules, 2023, 17, 28(3): 938.
- [12] Wilson JC, von Itzstein M. Recent strategies in the search for new anti-influenza therapies[J]. Curr Drug Targets, 2003, 4(5): 389-408.
- [13] Feng H, Chen G, Zhang Y, et al. Potential Multifunctional Bioactive Compounds from *Dyosma versipellis* Explored by Bioaffinity Ultrafiltration - HPLC/MS with Topo I, Topo II, COX-2 and ACE2[J]. J Inflamm Res, 2022, 15, 15:4677-4692.
- [14] Yu R, Chen L, Lan R, et al. Computational screening of antagonists against the SARS-CoV-2 (COVID-19) coronavirus by molecular docking[J]. Int J Antimicrob Agents, 2020, 56(2):106012.
- [15] Zhu J, Yan H, Shi M, et al. Luteolin inhibits spike protein of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) binding to angiotensin - converting enzyme 2. Phytother Res, 2023, 37(8): 3508-3521.