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Original Article

Effect of a Herbal Formulation on Infection Prevention and Immune Regulation in Children with Frequent Relapse Nephrotic Syndrome

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ABSTRACT

Objectives: This study aims to investigate the effect of a herbal formulation "Guyuan Tang" on preventing the recurrence of children with frequent relapse nephrotic syndrome and its influence on immune function. **Materials and Methods:** In all, 30 children with frequent relapse nephrotic syndrome were randomly divided into the Traditional Chinese Medicine (TCM) group and the control group. The control group was treated with conventional western medicine, while the TCM group was treated with Guyuan Tang for three months based a control group. The changes in the immune function indices before and after treatment were compared between the two groups, and the frequencies of infection and recurrence were compared after 12 months of follow-up. **Results:** The level of IgG protein, the proportion of CD3+CD4+T cells, CD4/CD8 and Treg cells in the TCM group increased after three months of Guyuan Tang treatment ($p < 0.05$), which showed statistical differences compared with the control group ($p < 0.05$). After one year of follow-up, no statistically significant difference was observed in the incidences of recurrence and infection between the two groups ($p > 0.05$), but the hospitalization rate and the average number of infections in the TCM group were significantly lower than those in the control group ($p < 0.05$). **Conclusion:** Guyuan Tang could reduce the frequency of infection in children with frequent relapse nephrotic syndrome, and regulating the proportion of Treg cells, CD4/CD8 and IgG may be one of its mechanisms.

Keywords: Frequent relapse nephrotic syndrome, Infection, Traditional Chinese Medicine, Immune, Children.

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INTRODUCTION

Primary Nephrotic Syndrome (PNS) is a common glomerular disease in children. Oral glucocorticoids are the accepted treatment for PNS. After initial glucocorticoid treatment, 10%–20% of PNS children show glucocorticoid resistance, and 76%–93% of PNS children who are sensitive to initial treatment relapse or even develop frequent relapse or glucocorticoid dependence.[1] Presently, the treatment of Frequent Relapse Nephrotic Syndrome (FRNS) is mainly to increase the dose of glucocorticoids or a combination with immunosuppressive agents, but this may cause metabolic disorders, immune dysfunction, and other side effects in children.[2,3]

Given the characteristics of FRNS in children, Professor Sheng Lixian established Guyuan Tang based on the three zang of lung, spleen, and kidney according to the theory of TCM. A herbal formulation "Guyuan Tang" has achieved a good curative effect in clinical applications. This study observed the effect of Guyuan Tang on the prevention of infection in children with FRNS and its effect on immunological indices and explored the mechanism of Guyuan Tang.

MATERIALS AND METHODS

Participants

In this study, 32 children aged 6–16 years with FRNS were selected between January 2016 and December 2018 at the First Affiliated Hospital of Zhejiang Chinese Medical University. The diagnostic criteria conformed to the 2021 KDIGO Guideline:[4] Recurrence criteria: for three consecutive days, morning urine protein changed from negative to (+++) or (++++), or 24 hr urine protein ≥ 50 mg/kg, or urinary protein/creatinine (mg/mg) ≥ 2.0; Frequency recurrence criteria: ≥2 recurrences within six

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months or ≥4 recurrences within 12 months during nephropathy. A flowchart of the study population is illustrated in Figure 1. The children were divided into the TCM group and the control group according to the random number Table method 1:1.

In the TCM group, there were nine males and seven females, with an average age of 8.20 ± 2.73 years and an average disease duration of 3.5 ± 1.0 years. In the control group, there were ten males and six females, with an average age of 8.53 ± 2.80 years; the mean disease duration was 3.5 ± 1.2 years. The general characteristics of the two groups are listed in Table 1. In the TCM group, there were 9 cases of minimal change nephropathy, 3 cases of focal segmental glomerulosclerosis, 3 cases of membranous nephropathy, and one case of mesangial proliferative glomerulonephritis. In the control group, there were 10 cases of minimal change nephropathy, 2 cases of focal segmental glomerulosclerosis and 4 cases of membranous nephropathy. This study was approved by the Ethics Committee of The First Affiliated Hospital of Zhejiang Chinese Medical University (2015-K-043-01).

Therapeutic Method

The treatment of the control group conformed to the 2021 KDIGO Guideline.[4] The TCM group was treated with Guyuan Tang based on the control group. During treatment and observation, children with respiratory tract infections were treated with symptomatic treatment, but traditional chinese medicine had to be stopped at that time. If recurrence occurred during the treatment period, the TCM group stopped the Guyuan Tang, and the two groups were given the corresponding treatment measures. If the withdrawal of Guyuan Tang was ≤2 weeks, the trial was continued, and the course of treatment was extended for two weeks. If Guyuan Tang was discontinued for more than two weeks, the trial would be stopped, and the case would be culled.

The composition of Guyuan Tang was Astragalus (15 g), Radix pseudostellariae (10 g), Atractylodes (10 g), Poria cocos (12 g), Saposhnikovia divaricata (6 g), licorice root (6 g), Phellodendron (6 g), Fructus amomi (6 g), and stigma of corn (30 g). The above herbs were decocted with 600mL water to obtain 300mL decocting juice per dose. The dosage was as follows: 6–9 years old, 100 mL each time; 10–12 years old, 120 mL each time; 13-16 years old, 150 mL each time. Take the juice twice a day (half an hour after breakfast and dinner). The child was revisited once every two weeks.

Clinical Observation

The children were followed up once a month for 12 months. The side effect, frequencies of recurrence and respiratory tract infection of the two groups were recorded during follow-up. Immunoglobulin levels (IgA, IgG, and IgM), complement (C3 and C4), white blood cell, neutrophil counts, and T-lymphocyte subsets in the peripheral blood were detected at the time of enrollment and three months after treatment, and the proportion of Treg cells was determined by flow cytometry. Before and after treatment, the liver and kidney function and blood lipid, including Serum creatinine (Scr), Aspartate Transaminase (AST), and Alanine Transaminase (ALT), levels were recorded for the two groups.

Statistical Analysis

The SPSS 22.0 software was used for the analysis. All the measurement data were in line with the normal distribution and homogeneity of variance, expressed as mean ± standard deviation (x±s), and a comparison between groups was performed using a *t*-test. Enumeration data were expressed as the number of cases and/or percentage (%), and a chi-square test was used to compare groups. $P < 0.05$ was considered statistically significant.

RESULTS

During the treatment, one case fell off in the TCM group and the control group because of a failure to return to the doctor on time, and 15 cases in the TCM group and the control group were included in the final statistics.

Comparison of immunological indices

Before treatment, no statistically significant difference in the humoral immunological indices was observed between the two groups ($p > 0.05$). After three months of treatment, the level of IgG in the TCM group was significantly higher than those before treatment ($p < 0.01$) and was higher than those in the control group ($p < 0.05$). No statistically significant difference in IgA, IgM, C3, and C4 was observed between the two groups before and after treatment ($p > 0.05$), as displayed in Table 2. The results of cell immunity showed that the CD3+CD4+T cell ratio, CD4/CD8 ratio and Treg cell ratio were significantly increased in the TCM group after treatment with Guyuan Tang ($p < 0.05$), and significantly different from control group ($p < 0.05$), as shown in Table 3.

Table 1: Comparison of characteristics between two groups.

Group	N	Male/Female	Age (Years)	Course (Years)	Frequency of Recurrence	Frequency of Infection
TCM	16	9/7	8.20 ± 2.73	3.5 ± 1.0	3.62 ± 0.61	3.12 ± 1.06
Con	16	10/6	8.53 ± 2.80	3.5 ± 1.2	3.52 ± 0.51	3.03 ± 1.13

Table 2: Comparison of humoral immunological indices before and after treatment (± s).

Group	Treatment	N	IgG (g/L)	IgA (g/L)	IgM (g/L)	C3 (g/L)	C4 (g/L)
TCM	Before	15	5.28 ± 1.59	2.33 ± 0.81	1.98 ± 0.45	1.12 ± 0.29	0.24 ± 0.08
	After	15	6.50 ± 2.06*△	2.14 ± 0.90	1.87 ± 0.64	1.16 ± 0.30	0.25 ± 0.08
Con	Before	15	5.01 ± 1.35	2.32 ± 0.95	2.20 ± 0.93	1.16 ± 0.37	0.26 ± 0.06
	After	15	5.05 ± 1.57	2.27 ± 0.93	2.25 ± 1.01	1.20 ± 0.32	0.27 ± 0.09

Note: *Compared with that before treatment, $p < 0.01$ △Compared with the control group after treatment, $p < 0.05$.

Table 3: Comparison of cellular immunological indices before and after treatment (± s).

Group	Treatment	N	WBC (10^9/L)	NE (10^9/L)	CD3+CD4+ T cell (%)	CD3+CD8+ T cell (%)	CD4/CD8	Treg (%)
TCM	Before	15	8.33 ± 1.60	4.09 ± 1.03	36.25 ± 2.42	27.08 ± 1.87	1.34 ± 0.05	4.77 ± 1.24
	After	15	8.27 ± 1.51	3.94 ± 1.04	38.54 ± 1.45*△	27.09 ± 1.25	1.43 ± 0.08*△	6.97 ± 2.14*△
Con	Before	15	8.45 ± 1.74	4.17 ± 1.30	36.29 ± 1.64	27.13 ± 1.31	1.34 ± 0.10	4.83 ± 1.17
	After	15	8.36 ± 1.44	4.17 ± 1.05	36.78 ± 1.26	27.12 ± 1.13	1.36 ± 0.09	5.13 ± 1.66

Note: *Compared with that before treatment, $p < 0.05$ △Compared with the control group after treatment, $p < 0.05$.

Table 4: Comparison of steroid response and side effects [n(%)].

Group	N	Hypertension	Hypertarachia	Insomnia	Cushing's syndrome	Steroid sensitivity	Steroid dependence	Steroid resistance
TCM	15	1 (6.7)	4 (26.7)	3 (20.0)	7 (46.7)	11 (73.3)	3 (20.0)	1 (6.7)
Con	15	2 (13.3)	6 (40.0)	6 (40.0)	10 (66.7)	8 (53.3)	4 (26.7)	3 (20.0)

Comparison of side effects of steroids

During treatment, the ratio of steroid sensitivity, steroid dependence and steroid resistance was 73.3%, 20% and 6.7% in the TCM group, and 53.3%, 26.7% and 20% in the control group. During the follow-up period, among the side effects of steroid, the proportion of hypertension, hypertarachia and insomnia in the TCM group was 6.7%, 26.7 and 20%, while the proportion in control group was 13.3%, 40% and 40%, as shown in Table 4. No statistically significant difference in Scr, AST, and ALT was observed between the two groups before and after treatment ($p > 0.05$), as demonstrated in Figure 2.

Comparison of frequencies of infection and recurrence

During the follow-up period, twenty-five children developed infections. In the TCM group, eleven children were infected, and the annual infection rate was 73.3%. In the control group, fourteen children were infected, and the annual infection rate was 93.3%. No statistically significant difference was observed between the two groups ($p > 0.05$). Two children in the TCM group were hospitalized for upper respiratory tract infection (bacterial infection). In the control group, two children were hospitalized for lung infections (bacterial infections) and five children for upper respiratory infections (three bacterial and two viral infections). The hospitalization rate of children in the TCM group was 13.3%, and the hospitalization rate of children in control group was 46.7%. There was a statistically significant difference between the two groups ($P < 0.05$). The annual average frequency of infection of the control group was 1.93 ± 1.49, and the annual average frequency of infection of the TCM group was 0.87 ± 0.99, which was significantly less than that of the control group ($p < 0.05$), as displayed in Figure 3 and Table 5.

In all, 19 children had recurrences during the follow-up. In the TCM group, eight children had a recurrence, including six cases of non-frequency recurrence and two of frequency recurrence, with an annual recurrence rate of 53.3%. In the control group, there were 11 recurrent children, including nine frequent recurrent children and two non-frequent recurrent children, with an annual recurrence rate of 73.3%. No statistically significant difference in the recurrence rate was observed between the two groups ($p > 0.05$). The annual infection frequency of recurrence in the control group was 2.53 ± 1.41, and the annual average frequency of recurrence in the TCM group was 0.80 ± 0.94, which was significantly less than that in the control group ($P < 0.05$), as illustrated in Figure 3 and Table 5.

Table 5: Comparison of the rates of infection and recurrence.

Group	N	Infection		Hospitalization		Recurrence	
		Yes	No	Yes	No	Yes	No
TCM	15	11 (44.0)	4 (80.0)	2 (13.3)	13 (86.7)	8 (42.1)	7 (63.6)
Con	15	14 (56.0)	1 (20.0)	7 (46.7)	8 (53.3)	11 (57.9)	4 (36.4)
P-values		0.330		0.048		0.450	

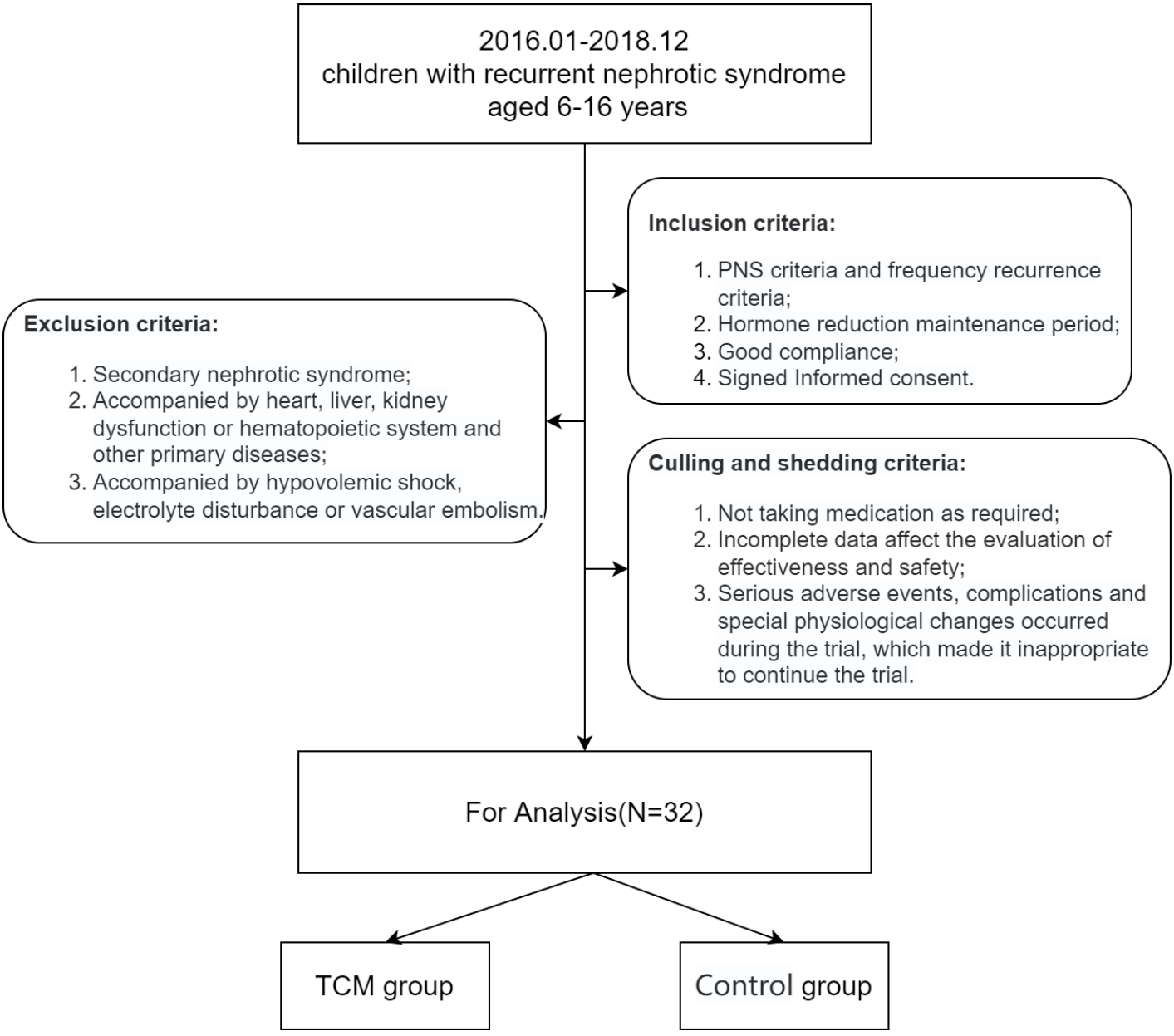

Figure 1: Flowchart of the study population.

DISCUSSION

At present, it is believed that the recurrence of PNS in children is related to the age of onset, infection, gene, and immune dysfunction.[5-8] Children with FRNS lose plasma proteins in urine for a long time, which easily leads to protein malnutrition, resulting in low immune function. In addition, some children often use immunosuppressive drugs, which aggravate immune dysfunction and make the children vulnerable to various infections.[9,10] It has been reported that infection is one of the main causes of recurrence in children with PNS and that the disorder of the immune internal environment is important pathogenesis.[11] The infection often leads to recurrence or frequent recurrence.

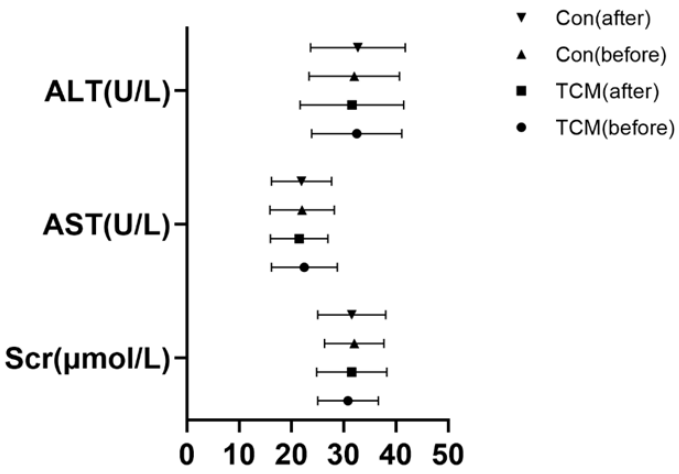

Figure 2: Liver and kidney functions before and after treatment between the two groups.

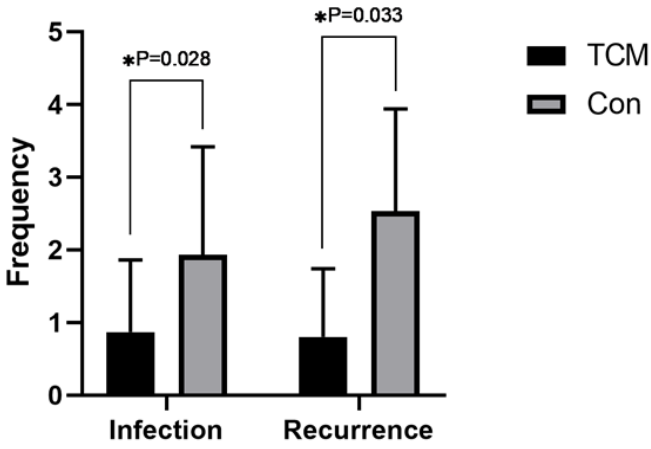

Figure 3: Frequencies of infection and recurrence between the two groups.

Therefore, preventing infection is very important to stabilize the disease and exert the effect of glucocorticoids.

According to the theory of TCM, the occurrence of infection is related to the deficiency of Qi, and the external manifestation of Qi is closely related to the immunological indices. Kidney is the origin of congenital constitution, and the spleen is the acquired foundation. When the spleen and the kidney are strong, the Qi will be sufficient. When there is sufficient healthy Qi inside, pathogenic factors have no way to invade the body. Guyuan Tang can nourish the spleen and the kidney, tonify Qi, and remove pathogens.

This study found that the annual frequencies of infection, hospitalization and recurrence in the TCM group were significantly lower than those in the control group, suggesting that Guyuan Tang could reduce or prevent infection, alleviate infection symptoms and reduce the recurrence of nephropathy. The rates of infection and recurrence in the TCM group were lower than those in the control group, but no statistically significant difference was observed. This could be attributed to the fact that the sample size of this study was small and the observation time was short. In terms of safety, no statistically significant difference in the liver and kidney functions was observed between the two groups, suggesting that Guyuan Tang had few side effects on the liver and kidney functions and was safe.

Immune dysfunction plays an important role in the pathogenesis of PNS.[12] The decrease in serum immunoglobulin affects the clearance of pathogens, which can lead to a decline in the body's resistance to disease, the occurrence of infection, and recurrence.[13] Different T-cell subsets can mediate cellular and humoral immunity by secreting cytokines and play an important role in the pathological changes of nephropathy.[14] IgG is the main component of immunoglobulin in serum, accounting for approximately 75% of the total immunoglobulin content in the serum. It has antiviral, neutralizing virus, antibacterial, and immune regulatory functions.[15-17] It was found that the serum IgG level of children with PNS is often decreased, while that of children with refractory nephrotic syndrome is significantly decreased, and the serum IgG level is positively correlated with the sensitivity of children to glucocorticoids.[18-20] In this study, the levels of IgG and CD3+CD4+ T lymphocytes of children with FRNS treated with Guyuan Tang was significantly higher than that of the control group, suggesting that its therapeutic effect on children with FRNS might be related to the improving immune function, thereby preventing infection and increasing hormone sensitivity.

Treg cells mainly secrete the transforming growth factor-β and IL-10, which are immunosuppressive and immunoincompetent, and play an important role in maintaining immune homeostasis.[21,22] Studies have found that an imbalance of Treg cells can cause a series of inflammatory and immune responses, resulting in pathological damage to the kidney. Compared with that in normal children, the proportion of Treg cells in children with PNS is significantly low.[23,24] In addition, the imbalance of Treg cells may be involved in the glucocorticoid resistance of children with PNS, and the proportion of Treg cells in children with hormone resistance is significantly lower than that in children that are glucocorticoid sensitive.[25,26] The results of this study revealed that the proportion of Treg cells in the TCM group was significantly higher than that in the control group after treatment, suggesting that Treg cells might be one of the targets of Guyuan Tang. Guyuan Tang might regulate the immune function and prevent or reduce the recurrence of children by regulating the Treg cells.

CONCLUSION

Guyuan Tang could regulate the proportion of immunoglobulin G, CD4/CD8 and Treg cells in children, reduce the infection frequency of children, and thus reduce the recurrence of nephrotic syndrome. However, the sample size of this study was small, and the observation period was short. A large-sample multicenter randomized controlled trial is required to further explore the effect of Guyuan Tang on the recurrence in children with FRNS.

STATEMENT OF ETHICS

This study was approved by the Ethics Committee of The First Affiliated Hospital of Zhejiang Chinese Medical University (2015-K-043-01). All participants signed informed consent.

FUNDING SOURCES

This study was financed by Zhejiang Provincial Natural Science Foundation of China (LGF21H270005).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

W.H. wrote the first draft of this paper, and T.B. revised this paper; W.Y. and W.Q. participated in collecting the samples; S.J. conducted the data analysis; All authors participated in the clinical design and approved the final manuscript.

ABBREVIATIONS

TCM: Traditional Chinese Medicine; **PNS:** Primary Nephrotic Syndrome; **FRNS:** Frequent Relapse Nephrotic Syndrome; **Treg:** Regulatory T cells.

SUMMARY

Primary Nephrotic Syndrome (PNS) is a common glomerular disease in children. After initial glucocorticoid treatment, 10%–20% of PNS children show glucocorticoid resistance, and 76%–93% of PNS children who are sensitive to initial treatment relapse or even develop frequent relapse or glucocorticoid dependence. Presently, the treatment of Frequent Relapse Nephrotic Syndrome (FRNS) is mainly to increase the dose of glucocorticoids or a combination with immunosuppressive agents, but this may cause metabolic disorders, immune dysfunction, and other side effects in children. A herbal formulation "Guyuan Tang", which was established by professor Sheng Lixian according to the theory of Traditional Chinese Medicine (TCM), has achieved a good curative effect in clinical applications for FRNS. This study investigated the effect of a herbal formulation "Guyuan Tang" on preventing the recurrence of children with FRNS and its influence on immune function. The results indicated that Guyuan Tang can regulate the proportion of immunoglobulin G CD4/CD8 and Treg cells in children, reduce the infection frequency of children, and thus reduce the recurrence of nephrotic syndrome. Thus, the herbal formulation "Guyuan Tang" may be an effective formulation for the treatment of FRNS.

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