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Original Article

Clinical significance of T lymphocyte subsets, immunoglobulin and complement expression in peripheral blood of children with steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome

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Abstract: Objective: To investigate the clinical significance of T lymphocyte subsets, immunoglobulin and complement expression in the peripheral blood of children with steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome (SDNS/FRNS). Methods: A prospective study was conducted on 285 children with nephrotic syndrome (NS). Among the 285 patients, 187 children had steroid-sensitive nephrotic syndrome (SSNS) and 98 children had SDNS/FRNS according to their sensitivity to hormones. Meanwhile, 50 healthy children in the same period were selected as the control group. Serum albumin (ALB), blood urea nitrogen (BUN), serum creatinine (SCr), estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), CD3+, CD4+, CD8+, immunoglobulin IgA, IgG, IgM and complement C3 and C4 were measured upon admission, and the content of urinary CD80 was also determined. Results: Compared with the control group, BUN, SCr, hs-CRP and IL-6 levels, urinary CD80, IgA, IgM and C3 in the SDNS/FRNS and SSNS groups were significantly higher, while ALB, eGFR, CD3+, CD4+, CD4+/CD8+, IgG and IgG/IgM were significantly lower (all $P<0.05$). Compared with the SSNS group, BUN, SCr, hs-CRP and IL-6 levels in the SDNS/FRNS group were significantly higher, while ALB and eGFR levels were significantly lower (all $P<0.05$). Compared with the SDNS/FRNS group, IgM in the SSNS group was significantly lower, while CD4+/CD8+, urinary CD80 and IgG/IgM were significantly higher (all $P<0.001$). Conclusion: Renal function decline and inflammatory response existed in children with NS. CD3+, CD4+, CD4+/CD8+ and IgG/IgM in peripheral blood were decreased, while IgA, IgM, C3 and urinary CD80 were increased. Moreover, renal function decline, increase of inflammatory factors, decrease of IgG/IgM and CD4+/CD8+ were more obvious in the SDNS/FRNS group.

Keywords: Nephrotic syndrome, T lymphocyte subsets, immunoglobulin, complement, clinical significance

Introduction

Nephrotic syndrome (NS) is a relatively common glomerular disease in that occurs in children, it is characterized by proteinuria, edema and hypoproteinemia, and its incidence is 757-984/10,000 showing an upward trend in high-risk areas, second only to acute nephritis [1-3]. NS in children can lead to a decline in quality of life and increased incidence of cardiovascular complications in the long term [4]. At present, the pathogenesis of NS is not clear, but previous study has found that abnormal immune cells and immune function were associated with NS [5]. Glucocorticoids are effective drugs for the treatment of NS in children. Most children with NS are sensitive to glucocorticoid therapy, which is called steroid-sensitive nephrotic syndrome (SSNS), however, nearly 25%-43% of children may experience relapse or hormone dependence after treatment, which is called steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome (SDNS/FRNS) [6]. SDNS/FRNS leads to prolonged use of glucocorticoids, which may have side effects and affect the growth of children [7].

Moreover, patients with protracted SDNS/FRNS may slowly develop end-stage renal disease [8]. If specific indicators can be found in children with NS, the prognosis of the children can be effectively evaluated, and early intervention is of great significance to improve the prognosis of patients and reduce the recurrence rate [9]. Therefore, this study investigated the T lymphocyte subsets, immunoglobulin and complement expression in the peripheral blood of children with SDNS/FRNS, so as to provide more clinical evidence for the occurrence and treatment of NS.

Materials and methods

Clinical data

A prospective study was conducted on 285 children with NS who were admitted to the Department of Pediatrics in Quanzhou First Hospital Affiliated to Fujian Medical University from January 2016 to December 2019. Among the 285 patients, 187 children had SSNS and 98 children had SDNS/FRNS according to their sensitivity to hormones. Meanwhile, 50 healthy children in the same period were selected as the control group. All patients were 1-14 years old, with an average age of 6.4±2.2 years old. This study was approved by the Ethics Committee of Quanzhou First Hospital Affiliated to Fujian Medical University. All the participants obtained the consent of their guardians and signed an informed consent.

Inclusion and exclusion criteria

Inclusion criteria: (1) Patients who met the NS reference of Evidence-based Guidelines for the Diagnosis and Treatment of Hormone-sensitive, Relapsed/dependent Nephrotic Syndrome in Children (2016) [6]; (2) Patients aged 1-16 years old; (3) Patients who did not take glucocorticoids after their initial diagnosis of NS.

Exclusion criteria: (1) Patients with congenital immune deficiency; (2) Patients with severe heart and lung diseases; (3) Patients complicated with malignant tumors; (4) Patients complicated with other autoimmune diseases; (5) Patients with incomplete clinical data.

Methods

The time of urine protein turning negative (the first time of urine protein turning negative after hormone therapy) of patients were observed and recorded, the time of initial recurrence (after complete remission achieved by hormone therapy, the time for the patient to have quantitative >3.5 g of urine protein for 24 h) was recorded, and the relevant indicators were also observed.

On admission, two tubes (5 mL each) of venous blood and morning urine (5 mL) were collected from patients on an empty stomach. Serum albumin (ALB), blood urea nitrogen (BUN), serum creatinine (SCr) and estimated glomerular filtration rate (eGFR) were determined by a fully automatic biochemical analyzer (Beckman Company, USA). High-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) were determined by enzyme-linked immunosorbent assay (ELISA) using an automatic microplate reader (Thermo Company, USA). Flow cytometry (Beckman Company, USA) was used to detect CD3+, CD4+, and CD8+ in peripheral blood, a protein analyzer was used to detect the content of IgA, IgG, IgM and complement C3 and C4 in patients, and the content of urinary CD80 was determined by ELISA (All the above kits were purchased from Shanghai Enzyme-Linked Biology Co., LTD., China).

Statistical analysis

SPSS 22.0 statistical software was used to analyze and process the data. The measurement data conforming to a normal distribution were expressed as mean ± standard deviation ($\bar{x}$ ± sd), and the measurement data that conformed to homogeneity of variance were analyzed with t test. The measurement data not conforming to a normal distribution was represented by M (P25, P75), and the measurement data that did not conform to a normal distribution and homogeneity of variance had a rank sum test. One-way ANOVA was used for comparison among multiple groups, and it was used to detect whether there were differences, if there were differences, Bonferroni method was further used to conduct post hoc comparison between two groups. $P<0.05$ was considered statistically significant.

Results

Comparison of clinical data

There were no significant differences in age and gender among the three groups ($P>0.05$).

Table 1. Comparison of clinical data (n, $\bar{x} \pm sd$)

Item	SSNS group	SDNS/FRNS group	Control group	χ^2/F/Z	P
Age (year)	6.3±2.1	6.5±2.0	6.5±2.3	0.374	0.688
Gender (male/female)	100/87	52/46	28/22	0.126	0.939
Clinical types				13.721	<0.001
Simple nephrotic syndrome	165	70			
Nephritic-nephrotic syndrome	23	30			
Time of urine protein turning negative (d)	10.8 (8.2-15.6)	25.1 (14.3-42.4)		5.289	<0.001
Time of initial recurrence (d)	11.4 (6.2-16.7)	3.5 (1.1-7.2)		4.215	<0.001

Note: SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

Table 2. Comparison of renal function and ALB ($\bar{x} \pm sd$)

Item	SSNS group	SDNS/FRNS group	Control group	F	P
BUN (mmol/L)	8.25±2.15***,#	10.36±3.02***	4.16±1.06	65.234	<0.001
SCr (μmol/L)	60.23±7.68***,##	80.65±8.36***	42.36±4.69	473.212	<0.001
eGFR (mL•min^{-1} (1.73m^2)$^{-1}$)	92.26±8.87***,##	80.21±7.21***	112.57±12.02	217.932	<0.001
ALB (g/L)	18.25±3.44***,#	14.29±2.72***	42.78±4.28	1291.112	<0.001

Note: Compared with control group, ***P<0.001; compared with SDNS/FRNS group, #P<0.05, ##P<0.01. ALB: serum albumin; BUN: blood urea nitrogen; SCr: serum creatinine; eGFR: estimated glomerular filtration rate; SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

Table 3. Comparison of inflammatory factor levels ($\bar{x} \pm sd$)

Item	SSNS group	SDNS/FRNS group	Control group	F	P
hs-CRP (mg/L)	13.45±3.78***,#	15.54±3.47***	4.23±1.07	191.715	<0.001
IL-6 (μg/mL)	374.36±36.78***,#	384.26±34.58***	173.36±20.98	760.821	<0.001

Note: Compared with control group, ***P<0.001; compared with SDNS/FRNS group, #P<0.05. hs-CRP: high-sensitivity C-reactive protein; IL-6: interleukin-6; SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

There were significant differences between the SSNS group and the SDNS/FRNS group in clinical type, time of urine protein turning negative, and time of initial recurrence (P<0.001). See **Table 1**.

Comparison of renal function and ALB

Compared with the control group, BUN and SCr levels in the SDNS/FRNS and SSNS groups were higher, while ALB and eGFR levels were lower (all P<0.001). BUN and SCr levels of the SDNS/FRNS group were higher compared with those of the SSNS group, while ALB and eGFR levels were lower compared with those of the SSNS group (all P<0.05). See **Table 2**.

Comparison of inflammatory factor levels

Hs-CRP and IL-6 levels in the SDNS/FRNS and SSNS groups were higher compared with those in control group (P<0.001), and hs-CRP and IL-6 levels in the SDNS/FRNS group were higher compared with those in the SSNS group (P<0.05). See **Table 3**.

Comparison of T cells in peripheral blood and urinary CD80

The CD3+, CD4+ and CD4+/CD8+ in the SSNS and SDNS/FRNS groups were all lower compared with those in control group, while the urinary CD80 was higher compared with that in control group (all P<0.001). CD4+/CD8+ and urinary CD80 in the SSNS group were higher compared with those in the SDNS/FRNS group (both P<0.001). There was no significant difference in CD8+ among the three groups (P>0.05). See **Table 4** and **Figure 1**.

Comparison of immune indices

Compared with control group, IgA, IgM and C3 in the SSNS and SDNS/FRNS groups were high-

Table 4. Comparison of T cells in peripheral blood and urinary CD80

Item	SSNS group	SDNS/FRNS group	Control group	F	P
CD3+ (%)	51.25±9.84***	53.56±11.35***	63.25±3.47	30.351	<0.001
CD4+ (%)	24.76±3.48***	22.29±3.26***	34.14±3.98	198.112	<0.001
CD8+ (%)	21.96±3.24	22.65±3.28	22.41±3.68	1.476	0.230
CD4+/CD8+	1.16±0.16***,###	0.97±0.11***	1.51±0.27	168.146	<0.001
Urinary CD80 (ng/mL)	7.72±1.24***,###	5.21±1.01***	3.69±1.14	307.812	<0.001

Note: Compared with control group, ***P<0.001; compared with SDNS/FRNS group, ###P<0.001. SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

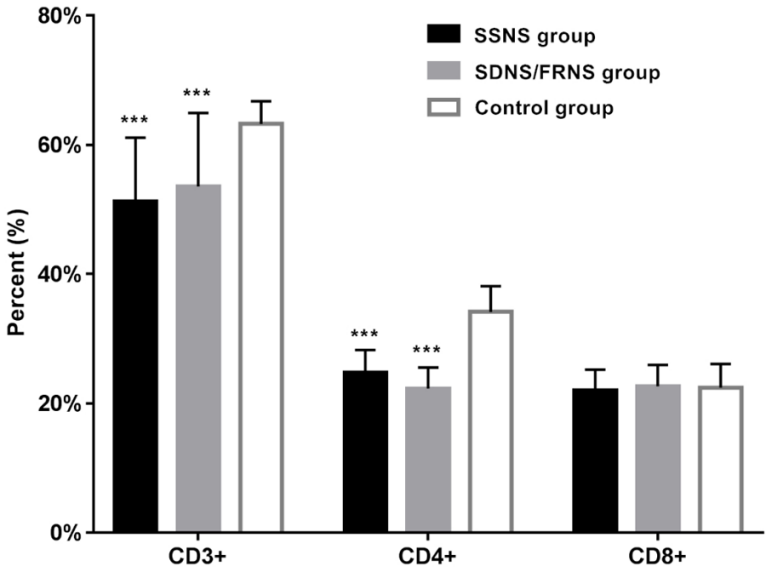

Figure 1. Comparison of T cells in peripheral blood. Compared with control group, ***P<0.001. SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

er, while IgG and IgG/IgM were lower (all P<0.001). Compared with the SDNS/FRNS group, the SSNS group had lower IgM and higher IgG/IgM (both P<0.001). There was no significant difference in C4 among the three groups (P>0.05). See **Table 5**.

Discussion

Nephrotic syndrome (NS) in children is a common disease seen in pediatrics. Most of the patients can control their condition to complete remission after treatment with glucocorticoids, and there is no recurrence after treatment. However, 25%-43% of the children may experience relapse or hormone dependence after treatment, and persistent proteinuria may occur because the disease cannot be effectively controlled [6]. Persistent proteinuria can aggravate kidney injury, which may lead to chronic renal impairment in patients, and chronic renal failure may occur in severe cases [10]. In this study, it was found that the renal function of children with SDNS/FRNS was significantly decreased in the early stage of the disease due to relapse and hormone dependence, and severe hypoproteinemia could increase the risk of complications, suggesting that the degree of kidney injury in children with SDNS/FRNS is more serious.

Inflammatory factors and immune dysfunction are closely related to the occurrence of NS in children. Inflammatory factors can be used as indicators to reflect the prognosis of children with NS [11]. Hs-CRP and IL-6 are specific inflammatory factor markers of the body, which can reflect the severity of inflammation in the body. The level of hs-CRP is correlated with the severity of the disease in children with NS. The higher the level of hs-CRP, the more serious the disease, and the two are positively correlated [12]. Hs-CRP and IL-6 can activate downstream inflammatory factors and affect the function of glomeruli and kidney tubules [12, 13]. This study also found that the hs-CRP and IL-6 level of children with SDNS/FRNS were higher compared with those of children with SSNS, which was consistent with the above studies. Immune dysfunction plays an important role in the occurrence and development of NS. T lymphocyte subsets are an important component to maintain normal immune functions of the human body, among

Table 5. Comparison of immune indices

Item	SSNS group	SDNS/FRNS group	Control group	F	P
IgA (g/L)	1.32±0.32***	1.35±0.36***	0.56±0.32	114.612	<0.001
IgG (g/L)	3.12±1.02	2.41±0.83*	6.04±1.49	207.311	<0.001
IgM (g/L)	1.51±0.35***,###	1.84±0.46***	0.89±0.24	107.521	<0.001
IgG/IgM	3.82±0.49***,###	2.87±0.56***	6.78±0.97	708.941	<0.001
C3 (g/L)	1.26±0.29***	1.29±0.31***	1.03±0.17	15.821	<0.001
C4 (g/L)	0.26±0.06	0.27±0.08	0.25±0.07	1.531	0.218

Note: Compared with control group, *P<0.05, ***P<0.001; compared with SDNS/FRNS group, ###P<0.001. SSNS: steroid-sensitive nephrotic syndrome; SDNS/FRNS: steroid-dependent nephrotic syndrome/frequently relapsing nephrotic syndrome.

which CD4+ and CD8+ play an important role in assisting and regulating immunity. The balance of CD4+ and CD8+ has an impact on immune function and is also closely related to the pathogenesis of NS [14, 15]. CD3+, CD4+ and CD4+/CD8+ are significantly decreased in the body of children with NS, suggesting that this may be related to the development and prognosis of diseases in children [16]. In addition, patients with NS have significantly decreased B lymphocytes, NK cells and NKT cells, and increased CD4+ [17]. In this study, it was found that CD3+, CD4+ and CD4+/CD8+ were decreased in children with NS, suggesting abnormal expression of T lymphocyte subsets in children with NS, which was consistent with the above research results. Since 70%-90% of children with NS have a pathological type of minimal change nephrosis (MCN) and are sensitive to glucocorticoid therapy, the common pathological type of hormone resistance and hormone dependence is focal segmental glomerular sclerosis (FSGS) [6]. The concentration of soluble CD80 in urine of MCN patients is significantly higher compared with that of the healthy control group [18]. Increased urinary CD80 level indicates increased sensitivity to hormones, which can be indirectly used as an indicator of hormone response sensitivity [19]. Further study found that there was a difference in urinary CD80 level between MCN and FSGS, which could be used as an indicator to distinguish between them [20]. This study found that urine CD80 in the SSNS group was higher compared with that in the SDNS/FRNS group and control group, which was consistent with the above research mechanism. Humoral immune disorders also exist in children with active NS. IgA, IgM and complement C3 are significantly increased in children with NS [21]. The ratio of IgG/IgM can reflect the sensitivity of children to hormones during treatment. When the ratio of IgG/IgM is greater than 3, it indicates that children are sensitive to hormones, otherwise it indicates hormone resistance or frequent relapse [22]. This study also showed that IgA, IgM and C3 were significantly increased and IgG/IgM was decreased in children with NS, and IgG/IgM in the SDNS/FRNS group was lower compared with that in the SSNS group, which was consistent with the above research results.

Limitations and prospects: This study was a single-center study, which can be further conducted a multi-center randomized controlled study. Since needle biopsy of kidney tissue was not performed in children with NS who were sensitive to hormone therapy, no pathological type analysis was performed in this study, which can be further improved in future study.

In conclusion, renal function decline and inflammatory response existed in children with NS. CD3+, CD4+, CD4+/CD8+ and IgG/IgM in peripheral blood were decreased, while IgA, IgM, C3 and urinary CD80 were increased. Moreover, renal function decline, increase of inflammatory factors, decrease of IgG/IgM and CD4+/CD8+ were more obvious in the SDNS/FRNS group.

Disclosure of conflict of interest

None.

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