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Comparison of serum cytokine/chemokine and FGF23 levels between children with steroid-sensitive nephrotic syndrome and those with frequently relapsing nephrotic syndrome : An exploratory case-control study

Hikari YAMADA, Shinji SUZUKI, Yasuyo KASHIWAGI, and Hisashi KAWASHIMA

Department of Pediatrics and Adolescent Medicine, Tokyo Medical University

Abstract

Background : Nearly 80% of Japanese children with nephrotic syndrome (NS) is steroid-sensitive NS (SSNS). Up to 50% of SSNS patients develop frequently relapsing NS (FRNS), and 50% of FRNS children have steroid-dependent NS (SDNS). However, some children do not experience relapses, and they recover completely. The predisposing factors of FRNS/SDNS and NS without relapse are unknown. Therefore, we measured the serum levels of cytokines/chemokines of patients in the active and remission stages of NS, to clarify whether changes in cytokine/chemokine levels are associated with FRNS/SDNS and NS without relapse, and whether there are any predisposing factors, as well as serum fibroblast growth factor 23 (FGF23), which is associated with the progression of chronic kidney disease in adults.

Methods : Fifteen children with primary NS who were admitted to our department between 2010 and 2019, and were followed up for a period between 2 and 10 years were enrolled in this study. The serum levels of 40 cytokines/chemokines were measured in these patients in the active and remission stages. Serum FGF23 was measured at the time of onset of NS. Three patients with chronic kidney disease owing to other causes were also analyzed.

Results : Serum levels of CXCL16 in the active stage, and CCL7, CCL15, and IL-16 in the remission stage were found to be lower in FRNS/SDNS patients than in NS patients without relapse. IL-16 level in the active stage showed a moderate negative correlation with level of proteinuria. The serum level of FGF23 in NS patients was significantly lower than that in patients with other kidney diseases, whereas there was no significant difference between FRNS/SDNS patients and NS patients without relapse.

Conclusion : The levels of CXCL16 in the active stage of disease, and of CCL7, CCL15, and IL-16 in the remission stage may be predisposing factors of the progression of pediatric NS to FRNS/SDNS. Further studies are required to identify predictive markers of FRNS/SDNS in children.

Introduction

Nephrotic syndrome (NS) is a disease caused by dysfunction of the glomerular filtration barrier, characterized by heavy proteinuria, hypoalbuminemia, hyperlipidemia, and edema, and is the most common glomerular disease in childhood. The characteristics of NS in children are heavy proteinuria (morning urine protein/creatinine ≥ 2.0 g/gCr) and hypoalbuminemia (≤ 2.5 g/dL)[1]. The incidence of NS in Japan is 6.5 in 100,000 children per year.

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Corresponding author : Hikari Yamada, MD, Department of Pediatrics and Adolescent Medicine, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan
TEL : +81-3-3342-6111 FAX : +81-3-3344-0643 E-mail : hikari5325@gmail.com

Nearly 80% of NS patients have steroid-sensitive NS (SSNS)[2)3)], and up to 50% of SSNS patients develop frequently relapsing NS (FRNS), which is defined as at least 2 relapses within 6 months of the initial presentation, or at least 4 relapses per year. SSNS patients often develop steroid-dependent NS (SDNS), defined as 2 consecutive relapses during tapering or within 14 days of cessation of steroid therapy. Approximately 50% to 60% of FRNS children have SDNS. These definitions are all from the International Study of Kidney Disease in Children (ISKDC) criteria[4)].

Although the pathogenesis of NS is not yet fully understood, some studies have suggested an important role of the immune system in triggering or maintaining NS, such as an abnormal response of T lymphocytes[5)6)] and increased local cytokine release[7)]. Cytokines are a group of proteins produced by several types of cells, which act as soluble mediators of intercellular signaling. Chemokines constitute a large family of low-molecular-weight cytokines that mainly act in the recruitment and activation of leukocyte subsets in some types of inflammation.

To avoid side effects caused by prolonged steroid therapy, children with FRNS/SDNS are usually treated with immunosuppressive agents. On the other hand, some children with NS recover completely without relapse after the initial treatment. As the prognosis for NS in childhood is favorable, with less than 5% progressing to end-stage renal failure[8)], there are few studies regarding their long-term outcomes. Studies from the 1980s reported that no more than 10% of children had additional relapses in adulthood[9)]. The risk factors of relapses during adulthood are thought to be a young age at onset, a high number of relapses during childhood, the use of immunosuppressive agents, and responding to steroid therapy[10)11)], whereas the predisposing factors of childhood FRNS/SDNS remain unknown. Therefore, it is important to clarify these predisposing factors, to provide the adequate initial treatment to children with FRNS/SDNS.

Fibroblast growth factor 23 (FGF23) is a circulating secretory protein with a molecular weight of 32 kDa, which is mainly expressed in osteocytes[12)]. Although plasma FGF23 is associated with the progression of chronic kidney disease (CKD) in adults[13)], there are no studies to date on serum FGF23 levels in children with NS.

Therefore, to investigate the usefulness of cytokine/chemokine levels, and FGF23 as predictors of the development of FRNS/SDNS, we performed an exploratory case-control study on pediatric patients in both the active and remission stages of disease.

Materials & methods

A total of 15 children (12 boys and 3 girls) with primary NS, who were admitted to our department between 2010 and 2019, with a follow-up duration between 2 and 10 years were enrolled in this study. The definitions of NS, FRNS, and SDNS are from the ISKDC criteria[4)]. The initial treatment comprised 2 mg/kg/day (maximum 60 mg/day) of prednisone given in 3 divided doses for 4 weeks, followed by treatment on alternate days for another 4 weeks. The daily dose was then tapered down for 4 to 7 months, and finally stopped.

The active stage of NS was defined as increased urinary protein excretion (Albustix ≥ 3+ for at least 3 consecutive days). The remission stage was defined as normal protein excretion (Albustix negative for at least 3 consecutive days). NS patients were tested during the active and/or remission stages. At the time of testing, none of the patients were taking any immunomodulatory drugs (e.g., cyclosporine A, cyclophosphamide, or rituximab), which could affect the immunological parameters being analyzed.

The serum levels of 40 cytokines/chemokines (IL-1b, 2, 4, 6, 10, and 16, CXCL-1, 2, 5, 6, 8, 9, 10, 11, 12, 13, and 16, CCL-1, 2, 3, 7, 8, 11, 13, 15, 17, 19, 20, 21, 22, 23, 24, 25, 26, and 27, CX3CL1, GM-CSF, MIF, IFN-γ, and TNF-α) were measured using a Bio-Plex suspension array (Bio-Rad Laboratories, Tokyo, Japan).

Serum FGF23 levels were measured using a RayBio®Human FGF-23 ELISA kit (RayBiotech Life, USA) in patients at the onset of NS. Three patients with other kidney diseases were also analyzed, i.e., 2 patients who were diagnosed with congenital renal hypoplasia, and 1 patient who was diagnosed as having reflux nephropathy (RN) with high vesicoureteral reflux and extensive renal scarring. The minimum detectable dose of human FGF23 was determined to be 0.3 ng/ml.

This study was approved by the Ethics Committee of Tokyo Medical University (study approval no. : T2020-0146), and written informed consent for participation was obtained from the participants' parents.

For the comparison between FRNS and SSNS, and between NS and other kidney diseases, data were analyzed using the Mann-Whitney U test and the Welch's *t*-test for continuous variables, and the Fisher's exact test for discrete data. In addition, the rate of change of cytokine/chemokine levels, defined as the difference in serum levels of cytokines/chemokines between the active and remission stages of a patient, divided by the serum levels of cytokines/chemokines in the active stage, were compared between patients with FRNS and patients with NS without relapse using the Welch's *t*-test. Correlations between proteinuria, serum albumin, and cytokines/chemokines in the active stage of disease were evaluated by

scatter plots and Spearman's rho. Two-sided *p*-values of less than 0.05 were considered to indicate a statistically significant difference between groups. All statistical analyses were performed using SPSS software version-27 for Windows.

Results

Patient characteristics

All FRNS patients were SDNS (FRNS/SDNS), and had relapsed after providing blood samples for this study. Table 1a shows the clinical characteristics of the patients with FRNS/SDNS and NS patients without relapse. Ten children (9 boys and 1 girl) had FRNS/SDNS with a median age of 4 (range : 1-10) years, 5 children (3 boys and 2 girls) had NS without relapse, with a median age of 8 (2-14) years. Their clinical data and treatment methods were retrospectively analyzed. There were no significant differences in proteinuria, albumin, T-cholesterol, or serum creatinine levels between the 2 groups, and the glomerular filtration rate in all patients was normal (data not shown). Table 1b shows the clinical characteristics of the NS patients and patients with other kidney diseases. Fifteen children (12 boys and 3 girls) with a median age of 3 (range : 1-14) years had NS, and 3 children (3 girls) with a median age of 0 (range : 0-6) years had CKD from other causes, i.e., 2 children had congenital renal hypoplasia, and 1 child had RN with high vesicoureteral reflux and extensive renal scarring. Statistically significant differences were observed in proteinuria, albumin, T-cholesterol, and serum creatinine levels between the 2 groups.

Cytokine/chemokine and FGF23 levels

Table 2 shows the levels of 40 cytokines/chemokines in patients in the active stage of FRNS/SDNS, and in NS patients without relapse. Only CXCL16 level was significantly lower in FRNS/SDNS patients than in NS patients without relapse. Table 3 shows the levels of 40 cytokines/chemokines in patients in the remission stage of FRNS/SDNS and in NS patients without relapse. CCL7, CCL15, and IL-16 levels in FRNS/SDNS patients were significantly lower than those in NS patients without relapse. We did not observe any statistical differences in the rate of change of cytokine/chemokine levels between FRNS/SDNS patients and NS patients without relapse, including levels of CXCL16, CCL7, CCL15, and IL-16 (Table 4a). IL-16 level significantly correlated with the level of proteinuria, whereas CXCL16, CCL7, and CCL15 levels did not correlate with proteinuria or serum albumin levels in the active stage of disease (Table 4b). Fig. 1a shows serum FGF23 levels in patients with NS and in patients with other diseases. Serum FGF23 level in patients with NS was significantly lower than that in patients with other kidney diseases. There was no significant difference in FGF23 level between FRNS/SDNS patients and NS patients without relapse (Fig. 1b).

Discussion

Few studies have focused on predictors of the recurrence of FRNS/SDNS in children. Mishra et al[14] reported that inadequate treatment of the first NS episode and the time to the first relapse being within 5.5 months might be predictive factors of FRNS/SDNS. Mishra et al[15] showed that the level of serum IL-13, which we did

Table 1a Clinical characteristics of patients with FRNS/SDNS and NS patients without relapse
Table 1b Clinical characteristics of NS patients and patients with other kidney diseases

a.

	FRNS/SDNS (n = 10)	NS without relapse (n = 5)	p-value
Age at onset (years)	4 (1-10)	8 (2-14)	0.22
Sex (boy : girl)	9 : 1	3 : 2	0.17
Proteinuria (g/day)	5.13 (0.23-10.70)	4.62 (1.87-8.12)	0.79
Albumin (g/dl)	1.6 (0.7-3.5)	1.7 (0.9-2.2)	0.82
Total cholesterol (mg/dl)	455 (255-671)	427 (283-536)	0.73
Serum creatinine (mg/dl)	0.45 (0.19-0.99)	0.44 (0.20-0.64)	0.93

b.

	NS (n = 15)	Other kidney diseases (n = 3)	p-value
Age at onset (years)	3 (1-14)	0 (0-6)	0.25
Sex (boy : girl)	12 : 3	0 : 3	0.03*
Proteinuria (g/day)	4.96 (0.23-10.70)	0.03 (0.00-0.06)	0.00*
Albumin (g/dl)	1.6 (0.7-3.5)	4.4 (4.2-4.6)	0.00*
Total cholesterol (mg/dl)	465 (255-617)	185 (134-214)	0.00*
Serum creatinine (mg/dl)	0.40 (0.19-0.99)	0.92 (0.31-12.21)	0.02*

Data are presented as the median (min–max). *$p < 0.05$ between the 2 groups
FRNS/SDNS : frequently relapsing nephrotic syndrome /steroid-dependent nephrotic syndrome

Table 2 Levels of 40 cytokines/chemokines in FRNS/SDNS patients and NS patients without relapse in the active stage of disease

	FRNS/SDNS (*n*=9)	NS without relapse (*n*=4)	*p*-value		FRNS/SDNS (*n*=9)	NS without relapse (*n*=4)	*p*-value
CCL21	40,906.0 (12,551.6-200,000.0)	46,809.8 (3,922.2-182,913.4)	0.88	IL-16	172.3 (0.0-383.7)	151.7 (0.0-194.9)	0.44
CXCL13	197.8 (28.7-388.3)	296.8 (11.3-492.4)	0.31	CXCL10	1,637.8 (93.3-2,551.3)	2,014.9 (41.1-11,993.7)	0.40
CCL27	596.0 (94.4-1,724.4)	308.0 (39.2-1,057.8)	0.34	CXCL11	197.4 (22.2-540.4)	111.2 (3.0-974.4)	0.88
CXCL5	3,140.8 (344.4-4,614.6)	2,820.4 (344.4-3,205.3)	0.49	CCL2	67.5 (34.3-355.6)	70.5 (16.2-80.9)	0.76
CCL11	351.0 (78.2-1,624.0)	304.0 (34.8-360.2)	0.40	CCL8	250.2 (84.7-1,185.7)	1,144.3 (42.6-2,103.4)	0.28
CCL24	2,063.5 (411.1-9,181.8)	2,361.4 (556.3-8,122.3)	0.88	CCL7	362.8 (216.2-615.6)	410.5 (204.0-478.9)	0.89
CCL26	399.1 (55.3-2,179.0)	573.8 (31.1-859.7)	0.54	CCL13	232.7 (114.6-701.8)	306.6 (15.6-657.7)	0.92
CX3CL1	1,166.2 (393.2-11,396.7)	7,394.6 (4,227.7-9,235.0)	0.44	CCL22	1,771.6 (164.1-3,353.0)	1,648.8 (887.5-2,621.0)	0.71
CXCL6	224.6 (85.6-601.1)	337.4 (33.4-363.3)	0.94	MIF	9,414.8 (1,838.2-157,523.1)	19,384.4 (2,507.9-43,986.9)	0.76
GM-CSF	0.9 (0.0-31.1)	0.0 (0.0-0.0)	0.08	CXCL9	164.3 (28.5-990.9)	199.7 (20.4-952.9)	0.94
CXCL1	797.8 (292.2-1,530.1)	860.2 (206.7-1,112.6)	0.67	CCL3	4.8 (2.8-24.4)	6.0 (2.7-8.2)	1.00
CXCL2	2,588.9 (455.5-8,285.5)	4,710.7 (157.5-20,631.0)	0.44	CCL15	6,048.9 (2,068.2-76,314.5)	9,100.0 (6,950.9-14,734.8)	0.35
CCL1	118.5 (69.2-492.8)	224.5 (51.1-312.1)	0.97	CCL20	22.4 (6.3-24.4)	15.5 (7.2-29.1)	0.69
IFN-g	35.0 (9.0-314.7)	48.2 (8.2-56.6)	1.00	CCL19	388.1 (252.1-1,096.0)	415.3 (289.9-543.0)	0.64
IL-1b	1.94 (0.90-6.15)	1.45 (1.10-1.56)	0.28	CCL23	281.2 (19.2-916.3)	294.0 (23.0-406.5)	0.50
IL-2	0.00 (0.0-5.89)	0.00 (0.0-2.09)	1.00	CXCL16	1,276.2 (231.0-3,253.2)	2,824.1 (2,566.5-3,762.1)	0.03*
IL-4	50.5 (22.1-109.8)	40.5 (17.4-48.6)	0.36	CXCL12	11,750.1 (3,579.7-26,536.6)	10,568.2 (5,429.3-21,396.7)	0.70
IL-6	36.6 (0.0-72.1)	77.7 (10.5-93.2)	0.20	CCL17	694.1 (146.0-1,072.5)	1,020.1 (43.6-2,405.2)	0.41
IL-8	11.9 (4.7-213.4)	16.9 (5.4-206.4)	0.76	CCL25	2,103.4 (1,150.5-5,942.2)	2,905.6 (530.6-3,459.6)	0.88
IL-10	4.1 (0.0-45.0)	0.0 (0.0-2.32)	0.09	TNF-α	21.1 (0.0-24.2)	9.9 (2.3-25.5)	0.54

Data are presented as the median (min–max). Concentrations of cytokines/chemokines are presented in pg/ml. *$p <$ 0.05 between the 2 groups
FRNS/SDNS : frequently relapsing nephrotic syndrome/steroid-dependent nephrotic syndrome

Table 3 Levels of 40 cytokines/chemokines in FRNS/SDNS patients and NS patients without relapse in the remission stage

	FRNS/SDNS (*n* = 9)	NS without relapse (*n* = 3)	*p*-value		FRNS/SDNS (*n* = 9)	NS without relapse (*n* = 3)	*p*-value
CCL21	25,727.2 (7,763.7-420,806.3)	28,938.9 (2,482.7-147,057.8)	0.93	IL-16	169.3 (79.2-287.7)	275.1 (204.9-376.1)	0.02*
CXCL13	206.9 (18.7-489.0)	172.5 (22.6-517.2)	0.68	CXCL10	1,292.1 (31.1-3,097.6)	1,410.7 (68.5-74,039.9)	0.64
CCL27	739.4 (451.3-1,219.3)	1,433.2 (405.3-2,925.1)	0.40	CXCL11	83.3 (7.1-532.1)	123.6 (14.8-2,664.3)	0.64
CXCL5	2,193.5 (362.4-196,197.0)	2,585.9 (2,394.6-3,974.1)	0.31	CCL2	60.1 (39.0-203.9)	112.5 (94.5-188.6)	0.27
CCL11	294.5 (47.7-403.9)	351.0 (134.3-1,495.6)	0.78	CCL8	329.9 (39.8-818.8)	695.3 (78.9-4,291.6)	0.42
CCL24	1,489.7 (403.9-8,941.9)	1,881.5 (1,262.1-3,656.6)	0.70	CCL7	312.9 (111.6-435.9)	420.8 (348.0-740.3)	0.03*
CCL26	288.6 (38.4-672.6)	696.3 (69.0-1,121.6)	0.19	CCL13	216.9 (65.8-527.8)	264.5 (159.7-918.8)	0.50
CX3CL1	3,294.5 (222.0-9,315.5)	2,868.7 (1,929.5-6,185.3)	0.87	CCL22	741.0 (298.4-1,386.5)	510.9 (234.6-5,572.1)	0.53
CXCL6	285.7 (121.1-816.0)	363.3 (123.8-403.4)	0.58	MIF	17,943.4 (1,995.9-82,020.0)	29,829.8 (7,119.8-77,891.0)	0.56
GM-CSF	0.0 (0.0-2.6)	3.0 (0.0-4.7)	0.12	CXCL9	149.6 (19.2-400.9)	267.4 (22.3-2,611.6)	0.42
CXCL1	872.5 (541.6-1,325.1)	952.1 (548.4-1,231.9)	0.87	CCL3	5.0 (2.0-18.9)	15.6 (2.6-16.0)	0.41
CXCL2	3,046.0 (378.1-7,197.7)	2,086.1 (2,078.1-7,984.9)	0.52	CCL15	5,048.0 (2,276.4-9,100.0)	9,100.0 (7,843.6-11,463.4)	0.04*
CCL1	264.6 (66.4-417.8)	296.4 (88.3-762.3)	0.41	CCL20	16.3 (2.7-29.1)	26.3 (6.5-50.3)	0.18
IFN-g	44.4 (5.9-84.0)	38.2 (13.4-134.4)	0.41	CCL19	326.3 (137.7-509.2)	404.4 (345.4-1,285.7)	0.35
IL-1b	1.80 (1.06-4.55)	3.55 (2.24-4.28)	0.12	CCL23	454.7 (4.1-800.0)	618.8 (19.2-907.4)	0.81
IL-2	0.0 (0.0-0.0)	0.0 (0.0-0.0)	-	CXCL16	1,494.1 (499.9-3,769.5)	1,376.9 (1,082.4-1,421.9)	0.50
IL-4	57.0 (41.4-86.9)	56.1 (50.6-129.5)	0.50	CXCL12	12,939.6 (2,928.9-40,893.3)	14,215.1 (5,146.9-39,146.3)	0.56
IL-6	18.1 (0.0-69.1)	28.7 (10.5-46.6)	0.96	CCL17	426.1 (48.7-1,440.0)	407.1 (362.4-1,513.6)	0.39
IL-8	23.1 (5.38-1,417.2)	32.0 (9.9-7,091.3)	0.78	CCL25	3,092.4 (854.2-5,113.8)	3,677.5 (1,736.9-6,418.3)	0.34
IL-10	3.4 (0.0-13.7)	11.9 (0.0-17.5)	0.45	TNF-α	10.9 (2.3-16.4)	10.8 (0.0-35.1)	0.69

Data are presented as the median (min-max). Concentrations of cytokines/chemokines are presented in pg/ml. *$p < 0.05$ between the 2 groups
FRNS/SDNS : frequently relapsing nephrotic syndrome/steroid-dependent nephrotic syndrome

not measure in this study, was higher in children with FRNS than in children with NS during the first episode. Furthermore, a recent study reported that children with FRNS/SDNS showed higher levels of high-sensitivity C-reactive protein and IL-6, and lower levels of CD4+/CD8+, than children with SSNS[16].

In the present study, we investigated the predictors of relapse using extensive serum immunological profiles, including that of chemokines. We found that the level of CXCL16 in the active stage of disease, and levels of CCL7, CCL15, and IL-16 in the remission stage were lower in FRNS/SDNS patients than in NS patients with-

Table 4a Rate of change in levels of 4 cytokines/chemokines between FRNS/SDNS patients and NS patients without relapse
Table 4b Correlations between levels of proteinuria, serum albumin, and 4 cytokines/chemokines in the active stage of kidney disease

a.

	FRNS/SDNS ($n = 8$)	NS without relapse ($n = 2$)	p-value
CXCL16	0.59 ± 1.98	− 0.62 ± 0.01	0.13
CCL7	− 0.35 ± 0.28	0.39 ± 0.94	0.46
CCL15	− 0.41 ± 0.50	0.13 NA*	NA*
IL-16	− 0.01 ± 0.51	1.85 NA*	NA*

b

	Proteinuria		Serum albumin	
	r	p-value	r	p-value
CXCL16	− 0.24	0.44	− 0.11	0.73
CCL7	0.06	0.86	− 0.23	0.45
CCL15	− 0.33	0.26	0.49	0.08
IL-16	− 0.613	0.03**	− 0.36	0.22

Data are presented as the mean ± SD. Concentrations of cytokines/chemokines are presented in pg/ml. *Statistical analysis was not possible as there was only 1 subject ; **$p < 0.05$ between the 2 groups
FRNS/SDNS : frequently relapsing nephrotic syndrome/steroid-dependent nephrotic syndrome ; NA : not applicable

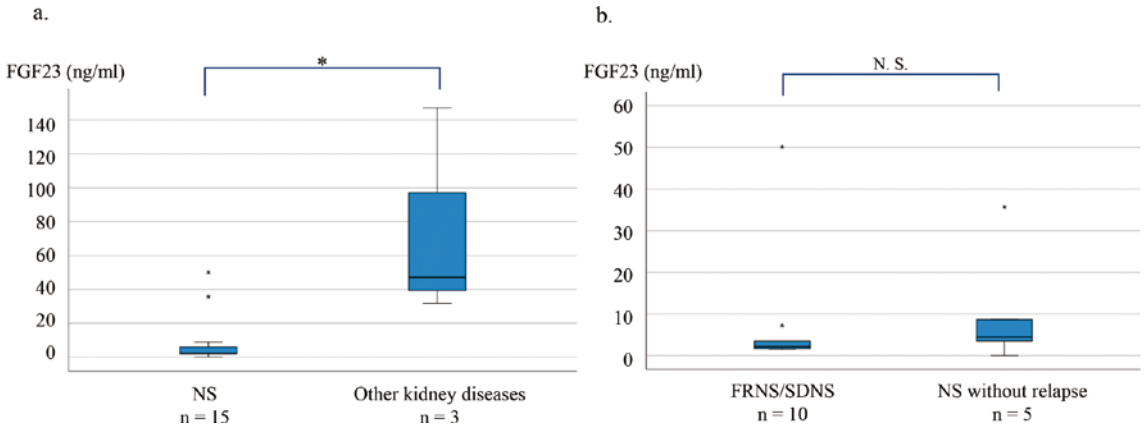

Fig. 1 Comparison of serum FGF23 levels between patient groups
(a) Comparison of serum FGF23 levels between patients with NS and those with other kidney diseases. (b) Comparison of serum FGF23 levels between patients with FRNS/SDNS, and NS patients without relapse. *$p < 0.05$ between the 2 groups, N.S. : not significant

out relapse. IL-16 level in the active stage showed a moderately negative correlation with level of proteinuria.

IL-16, which is a chemoattractant, is associated with various inflammatory processes. It was shown to be associated with inflammation-mediated renal injury in adults[17]. Huang et al[18] demonstrated that IL-16 facilitates immunocomplex deposition in blood vessel walls by stimulating B cells, leading to inflammation and kidney damage. Although patients with NS do not show the immunocomplex deposition, IL-16 may be associated with inflammation-mediated renal injury.

CXCL16 functions as both a chemokine and an adhesion molecule, and was found to be upregulated in response to kidney damage[19]. Zhen et al[20] demonstrated that increased CXCL16 levels strongly correlates with increased urinary protein levels, as well as the severity of immune reactions in children with active NS. A previous study showed that blockage of CXCL16 reduced glomerular damage and proteinuria by inhibiting monocyte recruitment in mice[21]. This suggests that

CXCL16 plays an important role in glomerular damage and its level correlates with disease severity.

CCL7 is a chemokine produced by B cells. Inaba et al[22] reported that CCL7 has the potential to facilitate neutrophil and monocyte recruitment to the injured kidney, and showed increased urinary CCL7 levels and CCL7 transcript levels in the kidney of adults with acute kidney injury (AKI). They also demonstrated that CCL7 blockade in mice reduced neutrophil recruitment to the kidney and ameliorated AKI.

CCL15 is a potent chemoattractant for monocytes *in vitro*, and induces the recruitment of neutrophils, monocytes, and lymphocytes in mice[23]. Richter et al[24] reported that CCL15 acts via CCR1, which is known as an important factor for monocyte recruitment in glomerular diseases, and showed increased serum CCL15 levels in adults with chronic renal failure.

Previous studies have indicated that lower levels of the above cytokines reduce the recruitment of neutrophils and/or monocytes to the kidney, leading to less damage to the kidney. Our results demonstrated that FRNS/SDNS patients express lower serum levels of IL-16, CXCL16, CCL7, and CCL15 than NS patients without relapse, which were different from the previous reports. The discrepancy with previous reports suggests that this is a characteristic immune profile of FRNS/SDNS patients.

The levels of CXCL16, CCL7, and CCL15 did not correlate with the levels of proteinuria or serum albumin, suggesting that they are not associated with the severity of pediatric FRNS/SDNS, but with the risk of recurrence. On the other hand, IL-16 levels in the active stage of disease negatively correlated with levels of proteinuria, and were significantly lower in the remission stage than in non-relapsing NS. This suggests that IL-16 is a relapse-related factor that is associated with disease activity. However, for IL-16, CCL7, and CCL15, their association with NS in childhood and adulthood has not been reported to date. Zhen et al[20] reported that CXCL16 in the active stage may be a marker of disease activity in NS, but their focus was on SSNS. Therefore, the interpretation of chemokines may be different in FRNS/SDNS, in which a different mechanism from NS has been suggested.

Moreover, previous studies on these cytokines/chemokines were performed in adult patients with kidney diseases, and hence the effects of aging should be considered when applying the results to children. It is possible that the differences in the results between adults and children with NS or FRNS/SDNS may have been a result of differences in their immune profiles.

Our study did not find a statistically significant difference in IL-6 level between the 2 groups, which was inconsistent with the previous study[16], as the timing of collection of the samples was different.

FGF23 is a bone-derived hormone, which controls blood phosphorus by increasing renal phosphate excretion and reducing 1,25-dihydroxy vitamin D3 production. Some reports demonstrated that FGF23 level is increased in both patients with acute and CKD, and is hence a strong independent risk factor for adverse renal outcomes and mortality[25][26]. In the present study, we analyzed serum FGF23 levels of NS patients at onset and 3 patients with CKD (2 caused by congenital renal hypoplasia and 1 by severe RN). Notably, we found that patients with NS had significantly lower serum FGF23 levels than patients with other kidney diseases. Yadav et al[27] reported that adult patients with untreated NS showed lower levels of FGF23 compared with healthy controls. They hypothesized that reduced levels of vitamin D caused by its urinary loss might result in a decrease in FGF23 synthesis. Our findings strongly support the possibility that FGF23 is involved in NS in children. Interestingly, Pukajło-Marczyk et al[28] demonstrated that patients with FRNS/SDNS receiving long-term prednisone treatment have higher levels of FGF23. Patients with FRNS/SDNS require long-term prednisone treatment, which may increase the risk of bone metabolism disorders. We measured FGF23 levels only at the time of onset of NS, and did not find a significant difference in between FRNS/SDNS patients and NS patients without relapse. Therefore, FGF23 level at the onset of NS is not a predisposing factor of FRNS/SDNS or NS without relapse.

There are several limitations to this study. The number of patients was very small, and they were from a single institution in a single area. As the characteristics of NS in children demonstrate ethnic differences, our findings cannot be generalized to all patients.

Further studies are necessary to understand the immune responses occurring in NS, and the identification of predisposing factors of childhood NS is expected to assist in the development of specific and individualized therapies, leading to the clinical improvement of patients and a more favorable disease prognosis.

Conclusion

In this study we analyzed the comprehensive serum immunological profiles of pediatric patients with FRNS/SDNS, and NS patients without relapse, towards understanding the predictive factors of recurrence in NS. The FRNS/SDNS group showed low levels of CXCL16 in the active stage of disease, and of CCL7, CCL15, and IL-16 in the remission stage, compared with the non-relapsing NS group. These characteristic profiles may provide clues towards identifying predictive markers of the progression of NS to FRNS/SDNS in children.

Conflicts of interest

The authors declare that they have no conflicts of interest associated with this study.

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小児における頻回再発型ネフローゼ症候群の免疫学的予後予測因子の検討

山田 ひかり　鈴木 慎二　柏木 保代
河島 尚志

東京医科大学病院小児科・思春期科

【要旨】 本邦における小児ネフローゼ症候群の発症頻度は 1 年間に 6.5/10 万人と欧米と比較し約 3 倍であり、小児腎臓病領域で重要な疾患である。小児特発性ネフローゼ症候群の 80% 以上はステロイド感受性ネフローゼ症候群であるが、約半数は頻回再発型ネフローゼ症候群やステロイド依存性ネフローゼ症候群（FRNS/SDNS）に移行する。一方で、初回エピソードのみのネフローゼ症候群（非再発群）も存在する。小児ネフローゼ症候群の病態生理は未だ明らかでないが、以前より T 細胞の免疫応答の異常や液性因子の関与が指摘されていた。本研究では、FRNS/SDNS と非再発群の活動期・寛解期の血清サイトカイン、ケモカインと、ネフローゼ症候群発症時の血清線維芽細胞増殖因子 23（FGF23）を測定し、FRNS/SDNS の予測因子について免疫プロファイルの観点から網羅的に検討した。対象は東京医科大学病院において 2010 年から 2019 年までに小児特発性ネフローゼ症候群と診断された 15 例で、後方視的検討を行った。本研究で FRNS/SDNS 群は非再発群と比較し、血清 IL-16、CXCL16、CCL7、CCL15 が低値を示し、小児 FRNS/SDNS を特徴づける免疫プロファイルであることが推測された。また、活動期の血清 IL-16 はタンパク尿と負の相関を示し、寛解期では非再発群と比較し有意に低く、IL-16 が疾患活動性と関連する再発関連因子であることが示唆された。FGF23 は骨細胞により産生される液性因子で腎近位尿細管のリン排泄とビタミン D を介した腸管のリン吸収を調節することで体内のリン濃度を調節している。血清 FGF23 値は他の腎疾患と比較しネフローゼ症候群で有意に低値を示し、特に FRNS/SDNS で低い値であったが、FRNS/SDNS と非再発群に差は見られず再発予測因子になり得なかった。筆者は血清 IL-16、CXCL16、CCL7、CCL15 は小児 FRNS/SDNS の予測因子として示唆され、また病態解明の一助となりうると結論づけた。

〈キーワード〉 ネフローゼ症候群、サイトカイン、ケモカイン、FGF23