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Children with Steroid-resistant Nephrotic Syndrome: a Single-Center Study

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Abstract

Background

Steroid-resistant nephrotic syndrome (SRNS) accounts for 10%-20% of all cases of idiopathic nephrotic syndrome. These patients are at risk of developing end-stage renal disease. The aim of this study was to determine the demographic characteristics, renal biopsy findings, response to immunosuppressive treatment, and prognosis in pediatric patients with SRNS.

Materials and Methods

This retrospective study included 31 patients diagnosed as primary SRNS. Age at first episode, gender, parental consanguinity, and familial history of nephrotic syndrome were recorded. Demographic characteristics, renal biopsy findings, response to immunosuppressive treatment and prognosis were analyzed, as were the number of and treatment of relapses, extra-renal manifestations and complications of disease and treatment. Data were analyzed using SPSS version 15.0

Results

Mean age at first episode of nephrotic syndrome was 4.1±2.9 years. At the end of the first immunosuppressive treatment cycle, 14 (51.8%) patients achieved complete remission, 4 (14.8%) patients achieved partial remission and 9 patients (33.3%) did not achieve remission. Analysis of the final status of the patients showed that 16 patients (51.6%) developed remission, 5 patients (16%) continued to have nephrotic range proteinuria and 10 patients (32%) developed chronic renal failure (CRF).

Conclusion

The treatment of SRNS remains controversial. Early genetic testing can help the inevitable immunosuppressive treatments which may not be effective and have several side effects. Calcineurin inhibitors and mycophenolate mofetil are known to be effective immunosuppressive drugs for treating steroid resistant nephrotic syndrome.

Key Words: Focal segmental glomerulosclerosis, Mutations, Steroid-resistant nephrotic syndrome.

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1- INTRODUCTION

The majority of children with idiopathic nephrotic syndrome (NS) respond to steroid therapy, but about 10%-20% have steroid-resistant nephrotic syndrome (SRNS) (1). In 50%-60% of children with SRNS the cause cannot be determined, whereas one third of patients have a single genetic defect that affects glomerular podocyte structure or function. Moreover, most patients with SRNS are unresponsive to immunosuppressive treatments (2). The most common mutations in patients with SRNS are in the NPHS1 gene that encodes nephrin, the NPHS2 gene that encodes podocin, and the WT1 gene that encodes transcription tumor suppressor protein. The most common observed histological lesion in SRNS patients is focal segmental glomerulosclerosis (FSGS) (2). SRNS in general and FSGS specifically are associated with a 50% risk of end-stage renal disease (ESRD) within 5 years of diagnosis in patients that do not achieve partial or complete remission (38). The aims of treatment are to correct proteinuria and preserve kidney function, but the optimal treatment method remains controversial. The present study aimed to determine the demographic characteristics, renal biopsy findings, genetic mutations, response to treatment, and prognosis in pediatric patients with SRNS.

2- MATERIALS AND METHODS

2-1. Definitions

SRNS was diagnosed in patients with idiopathic NS based on lack of complete remission despite treatment with oral steroids 60 mg/m²/day (maximum: 60 mg/d) for 8 weeks or 60 mg/m²/day for 4 weeks followed by Intravenous (IV) pulse methylprednisolone (MPZ) 30 mg/kg/day for 3 days (maximum: 1 g/ day). NS was defined as 24-h protein excretion >40 mg/m²/h, a urine protein/creatinine ratio (uPCR)>2 mg/mg or dipstick≥2+ and hypoalbuminemia ≥2.5 g/dL. Complete remission was defined as 24-h protein excretion <4 mg/m^2/h, uPCR <0.2 mg/ mg, or a trace-negative dipstick. Partial remission was defined as a ≥50% reduction in the basal proteinuria value or uPCR: 0.2-2mg/mg. Non-response was defined as a reduction in the basal proteinuria value <50% or a uPCR >2 mg/ mg. Relapse was defined as 3 consecutive days of ≥3+ proteinuria based on dipstick or a uPCR >2mg/mg in patients that were previously in remission. Chronic renal failure (CRF) was defined as an estimated glomerular filtration rate (eGFR) <80 mL/min/1.73 m² (irreversibly impaired kidney function). ESRD was defined as the need for renal replacement treatment or an eGFR <15 mL/min/1.73 m^2. Also, eGFR was determined by Schwartz equation (3).

2-2. Patients

This retrospective study included 31 patients diagnosed as primary SRNS. Patients with secondary SRNS were excluded from the study. Patient gender, age at first episode, parental consanguinity and family history of nephrotic syndrome were recorded. Renal biopsy was performed in 29 of the patients. Mutation analysis was performed in all patients using Sanger sequencing. All patients were investigated for NPHS2 and WT1 mutations, NPHS1 mutation was investigated in 4 patients that had congenital nephrotic syndrome (CNS), and LAMB2 mutation was investigated in one patient with CNS that was thought to have clinical Pierson syndrome. Patient demographic characteristics, renal biopsy findings, response to immunosuppressive treatment and prognosis were also analyzed. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Dr. Sami Ulus Children's Hospital Ethics Committee. Written informed consent was provided by the patients' families before the start of study.

2-3. Treatment

Immunosuppressive treatments, dosages and durations are shown in (Tables 1 and 2). Due to its side-effects, cyclophosphamide (CYC) was not administered for a period exceeding 12 weeks. After 6 months, patients that did not respond to cyclosporine A (CsA) were switched to mycophenolate mofetil (MMF) and if a response was achieved the treatment was continued for at least 12 months. Immunosuppressive treatment was not administered to the patients with CNS. In addition to immunosuppressive drugs,

oral prednisolone (at tapering doses) and renoprotective treatment (angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker, and anti-lipidemics) were administered when necessary in all patients. Patients with CRF were given supportive treatment and appropriate renal replacement therapy was administered in those with ESRD.

2-4. Statistical analysis

Data were analyzed using SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL). Data are expressed as frequency and percentage. Relationships between variables were analyzed via the chi-square test and Mann-whitney U test. The level of statistical significance was set at $P < 0.05$.

Table 1: The Tune-Mendoza protocol.

Weeks	IV Pulse MPZ*	Oral Prednisolone
1-2	30 mg kg^{-1} QWK**	-
3-10	30 mg kg^{-1} QWK	1.5 mg kg^{-1} eod***
11-18	30 mg kg^{-1} once every 2 weeks	1 mg kg^{-1} eod
19-50	30 mg kg^{-1} once per month	0.5 mg kg^{-1} eod
51-82	30 mg kg^{-1} once every 2 months	0.5 mg kg^{-1} eod

*MPZ: Methylprednisolone, **QWK: Every week; ***eod: every other day.

Table 2: Other immunosuppressive treatment protocols.

Immunosuppressive drugs	Dose	Dose interval (duration)
Cyclophosphamide	2 mg kg-1 day-1	Single dose (8-12 weeks)
Cyclosporine A	3-6 mg kg-1 day-1	2 doses (6-24 months)
Mycophenolate mofetil	1000-1200 mg/m2/day	2 doses (6-48 months)

3-RESULTS

The study included 31 (17 male and 14 female) SRNS patients. The male-female ratio was 1.2:1; the difference in the number of males and females was not significant ($P > 0.05$). Mean age at the first episode of SRNS was 4.1±2.9 years. The mean follow-up period was 63.06 ± 60.12 months. Parental consanguinity was noted in 13 (41.9%) of the patients and 5 (16%) patients had a family history of nephrotic syndrome. Patient demographic characteristics are shown in (Table. 3).

Renal biopsy was performed in 29 of the 31 SRNS patients and the findings were as follows: FSGS: n = 18 (62%); mesangial proliferation: n = 8 (27%); minimal change disease (MCD): n = 2 (6.8%); diffuse mesangial sclerosis (DMS): n = 1 (3.4%).
A mutation was noted in only 4 (12.9%) patients (Table.4), of which 2 had NPHS2 homozygous mutation. Mutation was detected in only 2 of the 4 patients with CNS: patient no. 1, who was diagnosed as Denys-drash Syndrome (DDS), had WT1 heterozygous mutation and patient no. 2,

who was diagnosed as Pierson syndrome, had a homozygous mutation in the LAMB2 gene. Extrarenal abnormalities were noted in 2 patients. Ambigous genitalia and bilateral Wilms tumors were noted in the patient with DDS, and eye abnormalities were observed in the patient with Pierson syndrome. Supportive treatment only (no immunosuppressive treatment) was administered to the 4 patients with CNS, whereas various immunosuppressive treatment protocols were administered to the remaining 27 SRNS patients. The response to the first immunosuppressive treatment, number of relapses, relapse treatment, final status of the patients at the last follow-up, renal biopsy findings and mutation analysis findings are shown at the end page of article (Table.5).

At least one immunosuppressive treatment protocol was administered to 27 of the patients with SRNS. The Mendoza protocol was administered to 6 patients of which 1 had partial remission and 5 had no response. CYC was administered to 8 patients, none of whom achieved remission. CsA was administered to 24 patients; 10 achieved complete remission, 4 had partial remission, and 10 had no response. MMF was administered to 9 patients of which 2 achieved complete remission, 1 achieved partial remission, and 6 had no response. In total, 14 (51.8%) of the 27 patients achieved complete remission, 4 (14.8%) achieved partial remission, and 9 (33.3%) did not have remission.

During follow-up, 12 relapses developed in 9 of the 14 patients that had achieved complete remission. In addition, 9 relapses occurred in 8 patients after the termination of CsA treatment. Treatment for all the relapses was 3 days of IV pulse Methylprednisolone (MPZ) 30 mg/kg/day followed by oral prednisolone. In 2 relapses complete remission was achieved with only pulse MPZ; for the other 10 relapses another immunosuppressive treatment (CsA or MMF) was required. In patients in which the duration of CsA treatment was administered for 2- year and in patients that did not respond to CsA for 6 months, the treatment was changed to MMF. At the time of the last follow-up 9 (51.6%) patients had achieved complete remission and 7 had achieved partial remission. Of the 15 (48.4%) patients that did not respond to immunosuppressive treatment, or had CNS, 4 of them continued nephrotic range proteinuria and 11 of them developed CRF. During follow-up period, CRF occurred mean in 29.4 ± 46.8 months. Among the 11 patients with CRF, 5 developed ESRD and subsequently received continuous ambulatory peritoneal dialysis (CAPD). Of the 5 patients with ESRD, 3 died due to CAPD and/or primary disease-associated complications and 2 previously had undergone renal transplantation. Of the 2 patients that underwent renal transplantation, 1 had CNS and a family history of nephrotic syndrome, but no mutation was found both in the patient and her parents. This patient renal biopsy showed mesangial proliferation. Renal transplantation from her father was performed 3 years ago, there was no post-transplant recurrence of disease and the patient is in complete remission. The other patient that had undergone renal transplantation had no mutation or family history of nephrotic syndrome. His renal biopsy showed FSGS. He underwent renal transplantation from his father 5 years ago and 1 month post transplantation disease recurrence developed, but following acute humoral rejection treatment the patient is now in complete remission. The most common complications in patients with SRNS were retarded growth and development (26%), osteoporosis (22.5%), cushingoid changes (16%), infections (16%) and hirsutism (13%). These complications were due to long-term, high-dose steroid use.

Table 3: Patient demographic characteristics

Gender (male/female)	Number (17/14)
Age at first episode	
0-4 months	4
4-12 months	1
13 months-5 years	20
6-12 years	6
Parental consanguinity	13 (41.9%)
Family history of similar disease	5 (16%)

Table 4: Genetic mutation findings

Patient no.	Genetic Mutations
1	WT1 gene, 9th exon c.1186G>C (p.Asp396His) heterozygous
2	LAMB2 gene, homozygous IVS4+2T > C donor splice site mutation
3	NPHS2 gene, 5th exon c.538G>A homozygous p.V180M protein change
4	NPHS2 gene, 4th exon 467delT homozygous L156fsX180

4- DISCUSSION

SRNS constitutes 10%-20% of all cases of idiopathic NS. In majority of children with SRNS the underlying cause cannot be determined, whereas one third of patients had single- genetic defects (2). The present study aimed to evaluate SRNS patients, in terms of demographic characteristics, renal biopsy findings, underlying genetic abnormalities, response to treatment, relapses, prognosis and complications.

Previous studies reported that NS was more common in males than females (4, 5). In a study of children in Nigeria by Anochie et al. (6) the male-female ratio was 1:1, whereas Mekahli et al. (7) reported a ratio of 1:4 and in the present study it was 1:2. NS emerges most often around the age of 2 years and 70%-80% of cases occur before age 6 years (8). With increasing age at the time of the first episode of NS, the frequency of FSGS increases and the response to steroid treatment decreases (9). In the present study mean age at first episode of NS was 4.1±2.9 years; 3.18 years in males, vs. 4.86 years in females. These findings are similar to those reported earlier in Turkey (10, 11). In the present study, the patients who have a mutation, age at onset of NS was lower. One third of SRNS cases are associated with a mutation in genes encoding proteins that negatively affect podocyte structure or function (12). The likelihood of a genetic mutation was significantly higher in SRNS patients with parental consanguinity or a family history of nephrotic syndrome (14). Among the present study's SRNS patients with a disease causing mutation, two had consanguineous parents and one had a family history of nephrotic syndrome.

Renal biopsy is recommended for histological diagnosis of children with SRNS and for determining treatment options and prognosis (15,16). The 3 most frequently seen histological findings are FSGS, mesangial proliferation and MCD (16). Several recent studies reported that as the FSGS rate increases, the steroid response rate decreases (17, 18). The best example of this was shown in a study by Balanszak et al. (19), in which 102 patients were evaluated during 2 distinct time periods (1986-1995 [period 1] and 1996-

2005[period 2]). Renal biopsy was performed in 28 patients during period 1 and in 44 patients during period 2. During period 2, the number of patients with MCD was lower and the number of those with mesangial proliferation and FSGS was significantly higher, as compared to period 1. In the present study mesangial proliferation and FSGS were found to be significantly high in our patients.

Genetic mutations are frequently seen in patients with CNS, hereditary NS and syndromic NS (20). Previous studies have recommended that to prevent unnecessary use of immunosuppressive drugs and the associated side-effects in SRNS patients, mutation analysis should be conducted, taking into consideration the age at onset of NS, renal histopathological findings and extra-renal anomalies (13,21). Risk factors for the development of SRNS associated with genetic causes include family history of nephrotic syndrome, the occurrence of proteinuria during the first year of life, and the presence of syndromic SRNS (22).

After diagnosis of SRNS, the first step is recommended to perform NPHS1, NPHS2 and WT1 gene mutation analysis in patients with CNS and NPHS2 and WT1 gene mutation analysis in patients without CNS. In syndromic patients with extra-renal findings specific genes, including LAMB2, SMARCAL1 and LMX1B, should be analyzed (23). Reported SRNS mutation rates varied by country (24). Genetic analysis of 110 patients with SRNS in Spain (22) showed that 33% of patients with a family history of nephrotic syndrome had a genetic mutation, vs. 25% of patients with a negative family history and that all 15 CNS patients had a mutation (NPHS1: n = 12; NPHS2: n = 1; WT1: n = 2). In the present study 4 (12.9%) of the 31 patients had mutation (NPHS2: n = 2; WT1: n = 1; LAMB2: n = 1); the 2 patients with NPHS2 mutation were homozygous. Polymorphism in the podocin gene was noted in 5 patients in the present study. SRNS patients with multi-drug resistance thought there is to be an underlying genetic pathology (24). Similarly, in the current study SRNS patients that were multidrug resistant, but not determined to have a genetic mutation or polymorphism, were thought to have an undetermined mutation. Among the present study, of 4 patients with CNS, 2 had a heterozygous WT1 mutation and a homozygous LAMB2 mutation; the same heterozygous mutations were in both patients' mothers. The father of the patient with Pierson syndrome could not undergo genetic analysis. When evaluated together with extra-renal findings, the patient with WT1 heterozygous mutation was diagnosed as DDS (25) and the patient with LAMB2 homozygous mutation was diagnosed as Pierson syndrome (26).

SRNS causes significant morbidity, the treatment of which is extremely difficult. Pulse IV MPZ was first recommended for the treatment of SRNS by Mendoza and Tune (27) in 1990; however, more recent studies have reported severe side-effects associated with high-dose, long-term steroid use. High dose and long-term steroid administration involves hospitalization and insufficient response; therefore, this treatment is no longer recommended (23). In the present study the Mendoza protocol was administered to 6 patients; of them, one had partial response and five had no response. In addition, multiple side-effect of long-term, high-dose steroid use were noted. Several studies reported that alkylating agents are highly toxic and not effective for obtaining remission in patients with SRNS (28, 29). In the present study CYC was administered to 8 patients, none of which achieved remission.

CsA has been shown to have a non-immunological, anti-proteinuric effect, in addition to its immunosuppressive effect (30); in support of this, CsA was shown to be effective in SRNS patients with NPHS2

mutation (31) and in patients with hereditary nephritis (32). Remission rates of 25%-87% were reported for CsA, which is commonly used drug for treating SRNS (33). Similarly, in the present study remission was achieved in 14 (58.3%) of 24 patients treated with CsA, but no response was obtained in two NPHS2 homozygous patients. Among patients treated with CsA, 50%-85% develop relapse post CsA treatment and 25%-50% of patients develop drug resistance (33). In the present study 12 relapses occurred in 9 of 14 patients that had achieved complete remission; 9 of these relapses occurred after termination of CsA treatment.

The literature includes only a few studies on MMF treatment in SRNS patients. A Brazilian study that included 52 SRNS patients treated with MMF reported that complete remission was achieved in 12 patients and partial remission in 19 (34). Gipson et al. (35) treated one group of SRNS patients with CNI and another group with MMF and oral dexamethasone. Complete or partial remission was noted in 33% of the patients in the MMF group and in 45.8% of those in the CNI group. In the present study, 9 patients were treated with MMF, with a remission rate of 33.3%.

In total, 15 (48.4%) of the present study's patients did not respond to treatment, NS continued in 4 patients, and CRF developed in 11 (35.5%). Mean time from first attack of NS to the development of CRF was 29.4 ± 46.8 months. This time period was similar to those previously reported from Turkey (10). ESRD was accounted for more than 10% of SRNS patients (36, 37) and in the present study 16.1% of the patients developed ESRD.

5-CONCLUSION

In conclusion, the optimal treatment of SRNS remains controversial. Identification of a genetic mutation is important factor for predicting responsiveness to immunosuppressive treatment. Early genetic testing may prevent the inevitable immunosuppressive treatments, which may not be effective and have several side effects. Despite the small patient population, the present study's findings show that age at the first episode of SRNS, parental consanguinity and family history of nephrotic syndrome did not have a significant effect on prognosis. The most important factor that affected morbidity and mortality in the patients with SRNS was the response to immunosuppressive treatment. CNI and MMF were effective immunosuppressive drugs; however, additional, large-scale prospective and controlled studies are needed to more clearly determine which are the most effectivness immunosuppressive treatments for SRNS.

6-CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

7-FINANCIAL SUPORT: No relevant financial relationship exists.

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Table 5: The response to the first immunosuppressive treatment and final status of the patients at the last follow-up

n.	1st ISD	2nd ISD	3rd ISD	Response to first treatment	Relapse frequency	Relapse treatment	Final status	Final treatment	Renal biopsy	Mutation
1.	Mendoza			CR	1	Pulse MPZ CNI - MMF	CRF	CAPD Renal tx	FSGS	-
2.	Mendoza- NR	CsA- NR	MMF	CR			CR		FSGS	NPHS2 polymorphism
3.	Mendoza- PR	CsA		CR			CR		FSGS	-
4.	Mendoza- NR	CsA- NR	MMF	NR			CRF	CAPD Ex.	FSGS	-
5.	CsA- NR	6 months pulse MPZ		CR			CR		FSGS	-
6.	CsA- NR	Mendoza- NR	CsA	NR			CRF	Supportivetreatment	FSGS	-
7.	CsA- NR	Mendoza- NR	CsA	PR			PR	CsA	FSGS	NPHS2 polymorphism
8.	CsA- NR	CsA		PR			PR	CsA	FSGS	-
9.	CsA- NR	CsA- NR	MMF	NR			NS	Supportivetreatment	MCD	NPHS2 homozygote
10.	CsA- NR	CsA		CR	1	Pulse MPZ	CR		Mesangial Proliferation	-
11.	CsA- NR	CsA- NR	MMF	PR			PR	MMF	FSGS	-
12.	CsA- NR	CsA		CR	2	Pulse MPZ CNI - MMF	PR	MMF	FSGS	NPHS2 Polymorphism
13.	CsA			CR			CR		Mesangial Proliferation	
14.	CsA			CR	1	Pulse MPZ	CR		Mesangial proliferation	NPHS2 polymorphism
15.	CsA			CR	1	Pulse MPZ MMF	PR	MMF	FSGS	-
16.	CsA- NR	MMF		NR			NS	Supportivetreatment	MCD	-
17.	CsA			CR	1	Pulse MPZ MMF	CR	MMF	Mesangial proliferation	-
18.	CsA			CR	1	Pulse MPZ CNI - MMF	NS	Supportivetreatment	FSGS	
19.	CsA- NR	MMF		NR			CRF	Supportivetreatment	Mesangial proliferation	NPHS2 homozygote
20.	CsA			PR			PR	CsA	FSGS	-
21.	CsA			NR			CRF	Supportivetreatment	FSGS	-
22.	CsA			NR			NS	Supportivetreatment	Mesangial proliferation	-
23.	CsA			CR	3	Pulse MPZ CNI	CR		FSGS	-
24.	CsA- NR	MMF		NR			CRF	Supportivetreatment	Mesangial proliferation	NPHS2 polymorphism
25.	CsA			CR	1	Pulse MPZ CNI	PR	CsA	FSGS	-
26	CsA- NR	MMF		NR			CRF	Supportivetreatment	FSGS	-
27.	CsA- PR	MMF		CR			CR		FSGS	-