

Focal segmental glomerulosclerosis variants in children with nephrotic syndrome

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Treatment

Abstract

Introduction

The aim of this study was to determine pathological variants of childhood focal segmental glomerulosclerosis and assess efficacy of prednisolone + methylprednisolone + cyclosporin A treatment.

Materials and methods

This retrospective cohort study included 134 native Kazakh children (3 months–17 years) with nephrotic syndrome hospitalised in the Nephrology Department of the Republic Children's Clinical Hospital from 2004 to 2011. Kidney biopsy and pathological investigations were performed in 38 nephrotic syndrome patients with proven steroid resistance.

Results

Focal segmental glomerulosclerosis was confirmed in 38 (28.4%) patients (treatment group). The main focal segmental glomerulosclerosis variants were glomerular tip lesions and not otherwise specified types. Historical (did not undergo kidney biopsy) controls were treated with cyclosporin A or alkylating agents. Prednisolone + methylprednisolone + cyclosporin A immunosuppressive therapy was highly effective, allowing complete remission in 88.9% patients with minimum side effects. Patients with the not otherwise

specified variant (*NPHS2* and *WT1* mutations) did not achieve remission. Combination cyclosporin A treatment was significantly more effective than alkylating agent treatment in steroid-resistant nephrotic syndrome patients without genetic mutations.

Conclusion

Tip lesions are predominant in childhood steroid-resistant nephrotic syndrome. Prednisolone + methylprednisolone + cyclosporin A therapy is safe and effective for childhood focal segmental glomerulosclerosis. Establishing a podocyte mutation profile is important to predict treatment outcome.

Introduction

Nephrotic syndrome (NS) constitutes the most common pathological glomerular condition in children, characterised by severe proteinuria (PU), hypoalbuminemia and edoema. The disease is routinely managed by steroid therapy¹. However, these patients may develop focal segmental glomerulosclerosis (FSGS), the major cause of childhood steroid-resistant nephrotic syndrome (SRNS). If NS persists after 6 weeks of prednisolone therapy, the steroid resistance of NS is confirmed. In the absence of adequate treatment, SRNS leads to end-stage kidney disease within 5–10 years. Therefore, early diagnosis of FSGS is essential to provide treatment alternatives and delay progression to end-stage renal disease.

Recent studies identified the podocyte as the predominant site of injury in FSGS². Experimental studies identified several factors contributing to podocyte damage: stretching¹,

viral infection, toxins³, immunological factors, mitochondriopathies^{4,5} and genetic mutations¹. Several genetic mutations of podocyte proteins have been clinically associated with congenital or infantile SRNS, including *NPHS1* (nephrin), *NPHS2* (podocin), *ACT4* (α -actinin-4), *PLCE1* (phospholipase C epsilon), *WT1* and *SMARCAL*⁶. However, few studies have addressed the impact of these mutations on treatment effectiveness in SRNS patients.

Since 1986, the calcineurin inhibitor cyclosporine A (CsA) has been used as a nonsteroidal alternative for the treatment of SRNS^{7,8}. However, the current protocols have not been optimised for children to avoid CsA-related neurotoxicity. Concurrently, prolonged treatment for several years with dose tapering is recommended. Although rare cases of SRNS with FSGS may develop resistance, almost 100% patients respond to CsA therapy^{9,10}. This study aimed to determine the pathological variants of childhood FSGS and test the safety and efficacy of combination immunosuppressive therapy comprising prednisolone (Pred) + methylprednisolone (MP) + CsA in children diagnosed with FSGS and investigate the impact of pathological childhood FSGS variants associated with genetic mutations in podocytes on treatment outcome.

Materials and methods

Subjects

A total of 134 NS patients who were hospitalised in the Nephrology Department of the Republic Children's Clinical Hospital 'Aksai' (Almaty) from 2004 to 2011 were

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included in this retrospective study. All the patients were native Kazakhs aged 3 months–17 years. The study was approved by the institutional Committee on Human Experimentation and performed in agreement with the Helsinki Declaration (JAMA 2000;284:3043–3049) and subsequent amendments. Informed consent was obtained from parents (guardians) of all the patients. Inclusion criteria were age below 18 years and NS. Exclusion criteria were rapidly progressive renal dysfunction and noncompliance.

Combination therapy with CsA

After confirmation of FSGS, all the children received intense 3-drug therapy: once-daily CsA (oral; 150 mg/m²), 3–6 pulses of MP (intravenous; 1 g/1.73 m²), and once-daily Pred (oral; 40 mg/m²). This regimen treatment was administered for 3–6 months, followed by dose tapering after NS remission, and the maximum duration was 6 years. Dosages were adjusted to maintain serum CsA concentrations at C₀ (80–120 ng/ml) and C₂ (700–1200 ng/ml), where C₀ is the baseline level and reflects dose efficacy and C₂ is the level measured 2 h after drug intake and reflects dose toxicity.

Sample collection

At present, 5 pathological FSGS variants are recognised: collapsing (COLL), cellular (CELL), glomerular tip lesion (GTL), perihilar and not otherwise specified (NOS). The type of FSGS in each patient with the condition was identified by kidney biopsy (Bx) analysis. Normally, Bx is not recommended for children with NS because of the high frequency (80%) of minimal change disease¹¹. However, it is indicated when NS persists after 6 weeks of steroid treatment, given the high risk of developing severe complications such as FSGS¹¹. In this study, Bx was performed for patients after ≥12 weeks of

unsuccessful steroid treatment. The procedure was performed using an automatic biopsy gun (Gallini S.R.L., Mirandola, Italy) and 16–18-gauge needles using ultrasound guidance under local or general anaesthesia. Two pieces of renal tissue were collected from each patient and fixed in 4% formaldehyde. Parafilm sections were stained for light microscopic evaluation (haematoxylin and eosin, Masson's trichrome, Periodic Acid–Schiff, methenamine silver and Congo red stains). Sections were also processed for immunohistochemical microscopy (IgA, IgM, IgG, C1q, C3, and kappa and lambda light chains) and electron microscopy. Pathological tissue analysis was conducted by experienced nephropathologists (Moscow and Saint-Petersburg, Russia).

Statistical analysis

The reported *p* values were calculated using natural logarithm-transformed intensities and Gaussian approximation to the *t*-distribution. Statistical

significance was established at *p* < 0.05.

Results

The mean period between the onset of NS and Bx was 11.7 ± 5.7 months (range: 3–32 months). The diagnosis of FSGS was confirmed in 38 children (16 boys and 22 girls; treatment group) with a mean age of 10.9 years (1.4–17 years; Table 1). The pathological variants of FSGS were identified as GTL (*n* = 18), NOS (*n* = 18) and COLL (*n* = 2). All the patients exhibited segmental glomerulosclerosis, and one-third of the patients had total glomerulosclerosis. They also presented significant focal (57.9%) or diffuse (26.3%) interstitial fibrosis and tubular atrophy. Most patients were diagnosed with IgM nephropathy (89.4%), as shown by immunolocalisation of IgM + C3 in the renal tissue.

The severity and prognosis of FSGS were determined on the basis of the extent of PU and decrease in the glomerular filtration rate (GFR). The mean baseline PU was 7.8 ± 3.5 g/day

Table 1 Histopathological glomerular and tubulointerstitial patterns in children with focal segmental glomerulosclerosis

Focal segmental glomerulosclerosis	Total	
	Absolute	%
<i>Variants of FSGS</i>		
Glomerular tip lesion	18	47.4 ± 4.5*
Not otherwise specified	18	47.4 ± 4.5*
Not otherwise specified associated with <i>NPHS2</i> and <i>WT1</i> mutations	4	10.5 ± 2.0
Collapsing	2	5.3 ± 2.0
<i>Glomerulosclerosis</i>		
Total	10	26.3 ± 4.0
Segmental	38	100.0
<i>Interstitial fibrosis/Tubular atrophy</i>		
Diffuse	10	26.3 ± 4.0
Focal	22	57.9 ± 4.5*
<i>Immunohistochemistry</i>		
IgM + C3	34	89.4 ± 2.8*
IgA + IgG + C3	2	5.3 ± 2.0
No staining	2	5.3 ± 2.0

*Significant difference; *p* < 0.05

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(range: 1.4–16 g/day), whereas GFR was 79.7 ± 22.6 ml/min (range: 31–110 ml/min). The symptoms of NS were associated with persistent haematuria (gross haematuria in 10 children) in 22 (57.9%) patients and arterial hypertension in 10 (26.3%) patients.

All the children were initially treated with Pred induction (60 mg/m^2 for 6–8 weeks) and maintenance therapy (40 mg/m^2 for 8 weeks, followed by tapered dosage). After confirmation of FSGS, the patients were divided into 3 groups: a treatment group, which included patients with FSGS ($n = 38$); a control (control A) group, which included patients who did not undergo Bx and were treated with CsA ($n = 30$); and another control group (control B), which included patients who did not undergo Bx and were treated with alkylating agents ($n = 66$), including cyclophosphamide ($n = 8$) and azathioprine ($n = 58$). The treatment group received combination therapy as described in the earlier section. Cyclophosphamide (oral; $2\text{--}3 \text{ mg/kg/day}$) was administered for 2–3 months with monitoring of the total blood count and PU, followed by maintenance therapy with $1\text{--}1.5 \text{ mg/kg/day}$ for 2 months. Azathioprine (oral; 0.2 mg/kg/day) was administered for 2 months, followed by maintenance therapy with 0.1 mg/kg/day for 2 months. Pred was administered together with alkylating agents in moderate dosages of $10\text{--}20 \text{ mg/m}^2/48 \text{ h}$. The treatment efficacy in the 3 groups was compared on the basis of PU and GFR levels measured before and after treatment. The mean treatment duration was 4.7 ± 2.8 years (2.2–7.0 years). The most significant impact on PU was observed in the treatment group, with a mean decrease of $7.7 \pm 2.1 \text{ g/day}$ (from $7.8 \pm 3.5 \text{ g/day}$ before treatment to $0.05 \pm 0.03 \text{ g/day}$ after treatment). In the control A

group, the mean decrease in PU was $5.8 \pm 1.6 \text{ g/day}$ (from $7.9 \pm 1.8 \text{ g/day}$ before treatment to $2.1 \pm 1.3 \text{ g/day}$ after treatment), which was significant ($p < 0.01$) compared with that in the treatment group. The control B group showed the least significant decrease in PU, i.e., $4.3 \pm 1.9 \text{ g/day}$ (from $6.8 \pm 2.4 \text{ g/day}$ before treatment to $2.5 \pm 1.1 \text{ g/day}$ after treatment). The most significant decrease in PU was achieved after CsA + MP + Pred combination therapy was initiated. This could be related to the presence of other histopathological patterns of NS such as membranoproliferative glomerulonephritis in patients in the control groups, where CsA was not so effective because of its podocytotropic effects with no strong effects on cellular proliferation.

After the follow-up period (4.2 ± 3.1 years), the mean decrease in PU in GTL patients was $8.9 \pm 2.1 \text{ g/day}$ (from $9.0 \pm 4.5 \text{ g/day}$ before treatment to $0.09 \pm 0.04 \text{ g/day}$ after treatment; $p < 0.001$), with complete remission of NS. In 7 patients with NOS without genetic mutations, the mean decrease in PU was $6.7 \pm 2.5 \text{ g/day}$ (from $6.8 \pm 3.2 \text{ g/day}$ before treatment to $0.6 \pm 0.1 \text{ g/day}$ after treatment), and complete remission of NS was achieved in these patients. Significant differences were observed between patients with GTL and those with NOS variants associated with genetic mutations ($p < 0.01$) as well as between patients with NOS variants associated with genetic mutations and those with NOS variants not associated with genetic mutations ($p < 0.05$). In patients with NOS variants not associated with genetic mutations, the mean increase in GFR was $5.3 \pm 2.2 \text{ ml/min}$ (from $92.9 \pm 15.4 \text{ ml/min}$ before treatment to $98.2 \pm 16.1 \text{ ml/min}$ after treatment). In patients with NOS variants associated with genetic mutations, the mean decrease in GFR was $21.5 \pm 5.1 \text{ ml/min}$ (from $75.0 \pm 7.1 \text{ ml/min}$ before treatment to

$53.5 \pm 12.3 \text{ ml/min}$ after treatment). In contrast, the mean increase in GFR was 28 ml/min (from 42 ml/min before treatment to 70 ml/min after treatment) in patients with COLL FSGS.

Discussion

In this study, 95% patients exhibited either the NOS or GTL variant of FSGS, whereas the remaining 5% patients exhibited COLL FSGS. The absence of the perihilar variant in our patients could be related to its secondary characteristics—arterial hypertension and obesity—which are more frequently encountered in adults. These data differ from the variant profile of a Chinese cohort of 212 children: NOS (40.6%), perihilar (11.8%), CELL (27.4%), COLL (5.6%) and GTL (14.6%)¹². In a cohort of 41 American children, the variant profile was as follows: NOS (44%), CELL (32%) and COLL (24%)¹³. Overall, these studies suggest that the NOS variant dominates in childhood FSGS, as also reported for adult patients¹⁴. The prognosis of FSGS patients is highly dependent on the type of variant, with reported 10-year renal survival rates of 80% for GTL, 50% for NOS and 30% for COLL¹⁴. Therefore, most patients in this study were considered to have a reasonable prognosis. However, most patients were diagnosed with IgM nephropathy, which is considered a high risk factor for SRNS. Therefore, these patients were excellent candidates for non-steroidal alternative treatments such as those including CsA.

The CsA + MP + Pred immunosuppressive therapy proposed in this study was significantly more efficient than conventional Pred treatment, with complete remission achieved in 88.9% patients. Treatment regimens including CsA require constant monitoring of CsA serum levels to avoid CsA-related nephrotoxicity¹⁵. This complication is generally reported for CsA treatments exceeding 3 years and

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in patients <5 years old. We observed 2 cases of acute CsA-related nephrotoxicity associated with high serum potassium and creatinine levels. These symptoms rapidly resolved after the dose of CsA was decreased. Notably, CsA-related nephrotoxicity is rare in children¹⁶. No other adverse effect was observed in our patients. The best therapeutic responses were observed in patients with GTL or NOS variants of FSGS not associated with genetic mutations. All of them achieved complete remission. Both patients with COLL FSGS achieved remission when mycophenolate mofetil was added to the immunosuppressive combination therapy. The weakest response to the immunosuppressive combination therapy was observed in patients with the NOS variant associated with podocyte mutations (*NPHS2* and *WT1*). After treatment, the immunosuppressive combination therapy was terminated because NS persisted, while renal function continued to deteriorate. PU decreased more significantly in the control A group than in the control B group. However, this result was less reliable than that for the treatment group because of the lack of histopathological diagnosis in the control groups.

Conclusion

This study demonstrates the safety and efficacy of CsA + MP + Pred therapy for the treatment of childhood FSGS. Furthermore, we showed that genetic mutations in podocytes significantly affect treatment success. This finding emphasises the importance of genotyping to determine the best course for treating SRNS in children diagnosed with FSGS.

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