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HISTOLOGICAL PATTERNS OF STEROID RESISTANT NEPHROTIC SYNDROME IN BULGARIAN CHILDREN: A SINGLE CENTRE STUDY

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Abstract

Nephrotic syndrome is the most frequent glomerular disease in childhood. Steroid resistant nephrotic syndrome (SRNS) represents about 20% of children with nephrotic syndrome. SRNS is one of the leading indications for performing renal biopsy in children. The objective of this retrospective study is to determine the histological patterns of SRNS in Bulgarian children and to compare them with the worldwide findings. The study included 49 patients with SRNS. All biopsies were performed at the Department of Pediatric Nephrology and Dialysis, University Children's Hospital, Sofia, Bulgaria between January 2004 and January 2020. The renal biopsies were examined histologically and with immunohistochemistry at the Pathology Department, Military Medical Academy, Sofia and Medical University – Sofia, Bulgaria. Twenty-eight boys (57.1%) and 21 girls (42.9%) were included in the study. The age at diagnosis ranged from 8 months to 212 months (17 years and 8 months) with a mean of 94 months (7 years and 10 months). Generalized edema was the most common presentation – 55.1%, followed by microhaematuria – 53.1% of cases. Histological examination revealed several different forms: Focal segmental glomerulosclerosis (FSGS) in 30.6% ($n = 15$), Mesangial proliferative glomerulonephritis (MesPGN) in 14.3% ($n = 7$), Membranous glomerulonephritis (MGN) in 12.2% ($n = 6$), IgM nephropathy in 12.2% ($n = 6$), Membranoproliferative glomerulonephritis (MPGN) type I in 8.2% ($n = 4$), C3 glomerulopathy 8.2%

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($n = 4$), Minimal change disease (MCD) in 4.1% ($n = 2$), IgA glomerulonephritis in 4.1% ($n = 2$), Diffuse mesangial sclerosis (DMS) in 4.1% ($n = 2$) and C1q nephropathy in 2.0% ($n = 1$). Although SRNS in Bulgarian children might represent a broad spectrum of glomerular diseases it could be attributed in approximately two/third of patients to four of them – FSGS, MesPGN, MGN, and IgM nephropathy.

Key words: steroid resistant nephrotic syndrome, histology patterns, pediatrics

Introduction. Nephrotic syndrome (NS) is the most frequent glomerular disease in childhood. It is characterized by high-grade proteinuria, hypoalbuminaemia and generalized oedema. Steroids have been used to treat idiopathic nephrotic syndrome since the early 1950s [1]. Children usually received prednisone 60 mg/day (maximum dose 80 mg/day) for four weeks at the onset of the disease. Patients with idiopathic nephrotic syndrome are classified according to their initial response to steroids into steroid-sensitive nephrotic syndrome (SSNS) or steroid-resistant nephrotic syndrome (SRNS). The definition of SRNS introduced by the International Study of Kidney Disease in Children (ISKDC) and used by the Arbeitsgemeinschaft für Pädiatrische Nephrologie (APN) is widely accepted: no urinary remission within 4 weeks of prednisone therapy 60 mg/m^2/day [2]. The other definition employed by the Society of French Speaking Pediatric Nephrologists states: no urinary remission following 4 weeks of prednisone 60 mg/m^2/day followed by three intravenous pulses of methylpredisolone [3]. SRNS represents about 20% of children with nephrotic syndrome [4, 5]. Different studies revealed that focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD) are the most common morphologic patterns seen in children with SRNS [6, 7]. The aim of this retrospective study is to determine the histological patterns of SRNS in Bulgarian children and compare them with the worldwide findings. In addition, the study will search for correlations between histological variants and disease course.

Material and methods. The material was retrieved from archival files of children with SRNS who underwent ultrasound guided percutaneous renal needle biopsy between January 2004 and January 2020 at the Department of Pediatric Nephrology and Dialysis, University Children's Hospital, Sofia, Bulgaria. The institution is a tertiary referral hospital. The study included cases of nephrotic syndrome (NS) with steroid resistance, either primary or secondary, that started at age of > 6 months and ≤ 18 years. Genetic forms were included. Commonly used definitions are:

1. Nephrotic range proteinuria (high-grade, $\geq$ 40 mg/m^2/h) or urine protein/creatinine ratio > 0.2 g/mmol;

2. Hypoalbuminemia, serum albumin ≤ 25 g/l;

3. Impaired renal function – GFR < 90 ml/min/1.73m^2;

4. High blood pressure – SBP or DBP $>$ 95th percentile;

5. Hematuria – red blood cells of more than 5/µL in a fresh uncentrifuged midstream urine specimen, or more than 3 red blood cells/high-power field in the centrifuged sediment from 10 ml of freshly voided midstream urine;

6. Complete remission – Urinary protein excretion ≤ 4 mg/m^2/h or 0-trace of protein on urine dipstick or protein/creatinine ratio < 0.02 g/mmol ($<$ 0.2 g/g) for three consecutive days;

7. Partial remission – a reduction in proteinuria more than 50% from baseline.

Histopathological assays: Light microscopic and immunohistochemical slides were made. For evaluation by light microscopy the slides were routinely stained with hematoxylin and eosin, PAS, 3 chrome Masson, Wilder's silver impregnation, Congo red. For immunofluorescence visualization DAKO kits with rabbit antihuman serum fits conjugated were used. Glomeruli were counted and examined for: number with global and/or segmental sclerosis; number of crescents; presence or absence of hypercellularity and matrix expansion; endocapillary proliferation. The results were expressed qualitatively by using the scale from 1+ to 3+. Tubulointerstitial changes were examined for the presence of: degenerative changes and tubular atrophy; interstitial fibrosis and/or interstitial inflammation. The immunofluorescence study included staining for: IgG, IgA, IgM, C1q, C3, C4 and fibrin.

Results. SRNS was diagnosed in 61 patients, but only in 49 of them renal biopsy was done: 28 boys (57.1%) and 21 girls (42.9 %) with a male:female ratio 1.3:1. The age at diagnosis ranged from 8 months to 212 months (17 years and 8 months) with a mean age of 94 months (7 years and 10 months). Most of the patients had primary idiopathic SRNS (95.9%), only two of them had secondary SRNS (4.1%). These are children with MGN (Common variable immune deficiency) and IgA nephropathy (Kostmann syndrome). The most common clinical symptom was generalized edema and it was presented in more than half of the cases – 55.1%. Microhematuria was also a very frequent finding – 53.1%. It was seen in 26 patients – nine out of 15 with FSGS (60%), four out of seven with MesGN (66.7%), four out of six with MGN (66.7%), three out of four with MPGN (75%), two out of four with C3 glomerulopathy (50%), two out of six with IgM nepropathy (33.3%), one out of two with IgA glomerulopathy (50%) and one with DMS. Only three children were with gross hematuria – two patients with MesGN and one with IgA glomerulopathy. Almost half of the cases were without a clear clinical picture – discrete edema or accidental finding of proteinuria – 44.9%. Hypertension was observed in 21 (42.8%) children before the beginning of steroid treatment. The rest of patients showed the age and sex-related normal values.

All the patients with MesPGN were hypertensive. There were three cases out of four with high blood pressure in the group with MPGN type I. Most of the patients were with normal eGFR 55.1% ($n = 27$), while hyperfiltration was noticed in 17 cases (34.7%). Five children (10.2%) were diagnosed with chronic kidney disease already at the onset of NS, unfortunately. Their renal biopsies have shown FSGS ($n = 4$) and MPGN type I ($n = 1$). There were ten patients with complications of the NS in our cohort. Four children were with acute kidney injury (AKI) during the first episode of NS – MesPGN ($n = 1$), MPGN ($n = 2$), MCD ($n = 1$), two were with thrombosis – C1q and IgM nephropathy and in three the disease was complicated by infection – MGN, IgA and C3 glomerulopathy. During the observational period the SRNS led to end-stage renal disease in nine patients (18.3%). Eight of them were with FSGS and one was with DMS. Four of them received renal transplant.

Histopathological findings. The mean number of glomeruli studied was 21 (range 5–40). FSGS was the most frequent histopathological finding, occurring in 15 out of 49 children (30.6%). MesPGN was the second most common histological form which was found in seven patients (14.3%). MGN and IgM nephropathy were diagnosed in six cases (12.2%), respectively. MPGN and C3 glomerulopathy were present in four children (8.2%) accordingly. DMS, MCD and IgA nephropathy were seen only in two children each (4.1%). One patient met the definition of C1q nephropathy. Histopathological findings in patients with SRNS are shown in Table 1.

Special attention was given to certain glomerular and tubulointerstitial patterns of the four most common histological findings. They are presented in Table 2.

It is expected sclerotic lesions to be mostly associated with FSGS – 39.9%, they were also found in 10.9% of patients with MGN. Sclerosis was almost absent in cases with MesPGN and IgM nephropathy. Mesangial expansion was very common in all four histological forms. It was noticed in 100% of MesPGN and

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Histopathological findings in patients with SRNS

Histological findings	Number of patients (%)	
Focal and segmental glomerulosclerosis (FSGS)	15	(30.6%)
Mesangial proliferative glomerulonephritis (MesPGN)	7	(14.3%)
Membranous glomerulonephritis (MGN)	6	(12.2%)
IgM nephropathy	6	(12.2%)
Membranoproliferative glomerulonephritis (MPGN) type I	4	(8.2%)
C3 nephropathy	4	(8.2%)
Diffuse mesangial sclerosis (DMS)	2	(4.1%)
Minimal change disease (MCD)	2	(4.1%)
IgA nephropathy	2	(4.1%)
C1q nephropathy	1	(2.0%)

Table 2

Glomerular and tubulointerstial patterns of the four most common histological forms

Histological pattern	Glomeruli				
	Segmental sclerosis (%)	Global sclerosis (%)	Crescent (%)	Mesangial expansion	
				grade	due to (%)
FSGS	17.7	22.2	0.07	1 + (21.4%)	Matrix (66.7%)
					Cells (33.3%)
				2 + (21.4%)	Matrix (66.7%)
					Matrix and cells (33.3%)
				Total 42.8%	Matrix (66.7%)
					Cells (16.6%)
					Matrix and cells (16.6%)
MesPGN	0	0.36	0	1 + (42.8%)	Matrix and cells (100%)
				2 + (57.2%)	Matrix (100%)
				Total 100%	
MGN	2.8	8.08	0	1 + (40%)	Matrix (100%)
				2 + (40%)	Matrix (100%)
				Total 80%	
IgM nephropathy	0	0	0	1+	Matrix (66.7%)
					Matrix and cells (33.3%)
				2+	Matrix and cells (100%)
				3+	Matrix (100%)
				Total 100%	

IgM nephropathy cases, in 80% of MGN and approximately in 43% of FSGS. Tubular changes and tubulointerstitial inflammation and fibrosis were remarkably high among children with FSGS and MGN (80.9% and 60%, respectively). It is interesting that only tubular degenerative changes were noticed in patients with IgM nepropathy, without any inflammation or sclerosis. Tubulointerstitial findings are described in Table 3.

Table 4 illustrates the immunohistochemical staining in the four most common histological forms. It is obvious that IgM and C3 depositions were dominant in children with FSGS (78.6% and 71.4%), but in fact IgG, IgA, C1q and C4 were also present. Staining for IgM and C3 were dominant as well in patients with MesPGN (85.7% and 71.4%), but there were no IgA and C4. All patients with MGN were positive for IgG, but in 80% there was a positive staining for IgA, IgM and C3 as well. C1q and C4 were also present, but staining for fibrin was negative. In all cases with IgM nephropathy there was a positive staining for IgM, but IgA, C1q and C3 were also found in some cases. It is interesting that in our patients with IgM nephropathy positive staining for fibrin was more frequent than in patients with FSGS.

Discussion. Approximately 20% of children with NS are steroid resistant [4, 5]. Renal histology provides important information in those patients. In our study the major causes of SRNS were FSGS (30.6%), MesPGN (14.3%), MGN (12.2%)

Table 3

Tubulointerstitial findings in the four most common histological forms

Histological form	Tubules and interstitium			
	Changes in the tubules	Inflammation (%)	Fibrosis (%)	Total (%)
FSGS	Degenerative changes and atrophy (92.8%)	71.4	78.6	80.9
MesPGN	Degenerative changes and atrophy (28.6%)	42.8	42.8	38.1
MGN	Degenerative changes and atrophy (100%)	20	60	60
IgM nephropathy	Degenerative changes and atrophy (100%)	0	0	33.3

Table 4

Immunohistochemistry staining in the four most common histological forms.
SEP – subepithelial; *SEN* – subendothelial; *MES* – mesangial; *INT* – interstitium; *BM* – basal membrane; *GM* – glomeruli; *VW* – vessels walls

Histological forms	IgG/ location	IgA/ location	IgM/ location	C1q/ location	C3/ location	C4/ location	Fibrin/ location
FSGS	14.3%/ SEP	21.4%/ SEP MES	78.6%/ SEP MES SEN	28.6%/ SEN MES SEP	71.4%/ SEN MES SEP *21.4% pos.(+) VW	7.1%/ MES	7.1%/ INT
MesPGN	14.3%/ BM	0%	85.7%/ SEP MES	14.3%/ SEP MES	71.4%/ GM *28.6% pos.(+) VW	0%	14.3%/ GM
MGN	100%/ SEP	80%/ SEP	80%/ MES SEP	60%/ SEP	80%/ SEP *20% pos.(+) VW	20%/ SEP	0%
IgM nephro-pathy	0%	33.3%/ SEP	100%/ MES SEP	50%/ MES SEP	33.3%/ MES *33.3% pos. (+) VW	0%	16.7%/ INT

and IgM nephropathy (12.2%). FSGS is the most common histological form. That finding might be explained by the fact FSGS could be a primary disease or secondary complication of other glomerular lesions and it is well known to be resistant to steroid therapy [8].

The prevalence of FSGS among children with SRNS in our study is comparable to that reported in South Africa [9] – 28.4% and Saudi Arabia [10] – 35%, lower than the observed in India [11] – 39.1%, USA [12] – 47% and Nigeria [6] – 39.1%. However, it is higher than that found in other studies in North America and Hong Kong [4,13] – 23%, North America and UK [13,14] – 5–20%. The lowest prevalence of FSGS in patients with SRNS was seen in the report of the Southwest Pediatric Nephrology Study Group (SPNSG) [15] – 7.1% (published more than 30 years ago). No clear gender difference in children with FSGS has been described in most reports [4,12]. In our study there is definitely male predilection (nine boys vs. six girls). It has been shown that the prevalence of FSGS among children with SRNS is increasing with age. Although in two thirds of children NS appears before 6 years of age, only a minority of younger children exhibit FSGS. Usually it is a more common histology finding in the older group. It has been reported that the frequency of FSGS in NS patients presenting before 6 years of age is less than 10%, but increases to 20–50% or more in adolescents [13,15]. In our study eight of the patients with FSGS (53.3%) were diagnosed before the age of 6 years (four boys and four girls). The remaining seven patients developed disease in adolescence (five boys and two girls).

The prevalence of MesPGN in children with SRNS in our study is 14.3%. There is a wide range of reported frequency of MesPGN in the literature. For example, in India [16] it is estimated to be 35.2%, but approximately 20% in China [17]. A study from Egypt [18] has found very low prevalence – 1.9%. It was also low in Saudi Arabia [10] – 8.7%. Filler et al. [14] analyzed a 17-years database covering a 275 000-child population with mandatory referral. They have reported a 5% prevalence of MesPGN in renal biopsies.

According to the same survey the prevalence of MGN is 1.9%. Most authors have also shown an incidence rate close to 2%. Chen et al. [19] declared an incidence of 2.8% in their USA study. We found a prevalence of 10.2% in our study, which is very high. IgMN have accounted for 41% of renal biopsies in a single hospital survey in USA [20]. The prevalence in Saudi Arabia [10] is 23%. The prevalence in our study was 12.2%. Mesangial hypercellularity was revealed in 100% of patients with MesPGN and IgM nephropathy and in 80% of patients with MGN. We could speculate that this is an important factor leading to steroid resistance in these patients. Remarkable tubulointerstitial changes and fibrosis were observed in FSGS (80.9%), but also in MGN (60%). It was reported that patients with significant tubulointerstitial fibrosis and/or glomerulosclerosis would not get any benefit from steroid therapy and they were more likely to be harmed from various side effects.

Conclusion. Although SRNS in Bulgarian children might represent a broad spectrum of glomerular diseases it could be attributed in approximately two/thirds of patients to four of them – FSGS, MesPGN, MGN, and IgM nephropathy.

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