ORIGINAL ARTICLE

Histopathological Features of Primary Nephrotic Syndrome in Children

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ABSTRACT Renal biopsy was performed on 28 out of 50 children with primary nephrotic syndrome encountered during the period January 1994 - December 1995. Light microscope (LM) and immunofluorescence microscope (IM) examinations were performed on all biopsy specimens. LM examination indicated minimal changes (MC) in 13 cases (46.4%), focal segmental glomerulosclerosis (FSGS) in 10 (35.7%), membranous glomerulonephritis (MG) in 2 (7.1%), mesangial proliferative glomerulonephritis (MPG) in 7 (7.1%), and membranoproliferative glomerulonephritis (MPGN) in 1 (3.6%). On IM examination, immunoglobulin deposit was not detected in any MC patients, whereas in FSGS, IgG, IgM, C3 and fibrinogen deposits were found. In the MG group, IgG deposition was detected in one case. In the MPG cases, depositions of IgA, IgG, IgM, C3 and fibrinogen were detected and in the case of MPGN, deposits of IgM and C3 were found. Regarding to response to steroid treatment in the MC group, there was a significant difference between the steroid sensitive and steroid insensitive ($p<0.05$). For the FSGS abnormality in the steroid treatment of the insensitive patients, there was found significant difference with the steroid sensitive abnormality ($p<0.05$). In conclusion, nephritic symptoms (hematuria, proteinuria, azothemia) are possibly the non minimal group and hence, it would be necessary to carry out renal biopsy to prove this. **[Paediatr Indones 1997; 37:20-28]**

Introduction

Nephrotic syndrome (NS) is a clinical term for condition characterized by edema massive proteinuria, hypoalbuminemia, and hypercholesterolemia. In children, laboratory results consistent with nephrotic syndrome include proteinuria more than 1 g/24 hours, hypoalbuminemia <2.5 g/100 ml, and/or hypercholesterolemia > 250

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mg/dl.[1,2] Percutaneus biopsy has developed into a diagnostic tool, accompanied by a more thorough histopathological examination technique using light microscope, immunofluorescence microscope, as well as electron microscope. The results of kidney biopsy examination constitute a good guide in determining the prognosis, and in a number of cases, for determining treatment.[3] Indications for the need of renal biopsy in nephrotic syndrome patients are NS who have not received steroid treatment and the presence of one of the following accompanying symptoms: hematuria, hypertension, decreased renal function, or age less than 6 months. Apart from this, renal biopsy is also carried out on NS patients, when after receiving steroid treatment for 4 weeks, proteinuria is still found. It is suspected that the patient belongs to the steroid dependent or resistant. We review the results of renal biopsy examination of primary nephrotic syndrome in children. Association between histopathological features and result of treatment will also be discussed.

Materials and Methods

The study was carried out from January 1994 to December 1995, on child primary nephrotic syndrome patients treated in the pediatric ward of Sardjito General Hospital in Yogyakarta. On all patients fulfilling the primary NS diagnostic criteria in accordance with their indications, percutaneous renal biopsy was carried out with the guidance of USG, and using Tru Cut No.2N2704T (Batter) needle. Secondary NS patients, i.e., NS caused by various primary diseases such as systemic lupus erythematosus, Henoch-Schonlein purpura, endocrine diseases, and others, were not included in this study. Renal biopsy preparations for light microscopic examination were partly done in the Anatomical Pathology Section of Gadjah Mada University, Yogyakarta, and partly carried out in the laboratories of the Medical School, University of Indonesia, Jakarta. Preparations for immunofluoresence microscope (IM) examination were entirely performed in the Department of Anatomical Pathology, Medical School, University of Indonesia, Jakarta.

Light microscope study

The entire renal biopsy preparations were fixed in buffered 10% formalin solution, processed manually, mounted in a paraffin block, and subsequently sectioned into 2 micron thick. The preparations were then rinsed with hematoxylin and eosin (HE), periodic acid schiff (PAS), and Goldner trichrome. In several cases, rinsing with periodic acid silver metheamin (PSAM) was added.

Immunofluorescence microscope study

Renal biopsy preparation received in a fresh condition, were rapidly frozen ("snap frozen") using "dry ice", and OCT compound was used a substance to implant tissue;

the frozen tissue was subsequently sectioned into 4-6 micron thick using cryostat microtome. These thin sections were then washed with PBS solution for 10 minutes to remove remaining OCT compounds. Direct anti fluorescence substance technique was used for immunofluorescence examination on kidney tissue, then thin sections of the tissue were coated with rabbit anti human IgG, IgM, IgA, C3 and fibrinogen, which had been fixed into FITC (fluorescein isothiocyanate). Tissue which had been coated with the conjugate was then incubated in a moist vessel for 30 minutes, after which it was washed in a PBS solution with 3 times changing of the washing solution, each for 5 minutes. Subsequently, PBS-glycerine solution was applied to the preparation, then the preparation covered with the glass lid and examined under fluorescence microscope. In this study, biopsy results which were not representative, or were not produced by examination with the two microscopes, were not included in this report.

Results

During the period of 2 years, from January 1994 to December 1995, 50 new nephrotic syndrome patients were obtained. Of this number, 30 patients fulfilled the criteria for biopsy to be carried out according to indications, but only 28 patients could actually be evaluated, consisting of 20 (or 71.4%) male children and 8 (or 28,6%) females, giving a male to female ratio of 2,4:1. See Figure 1.

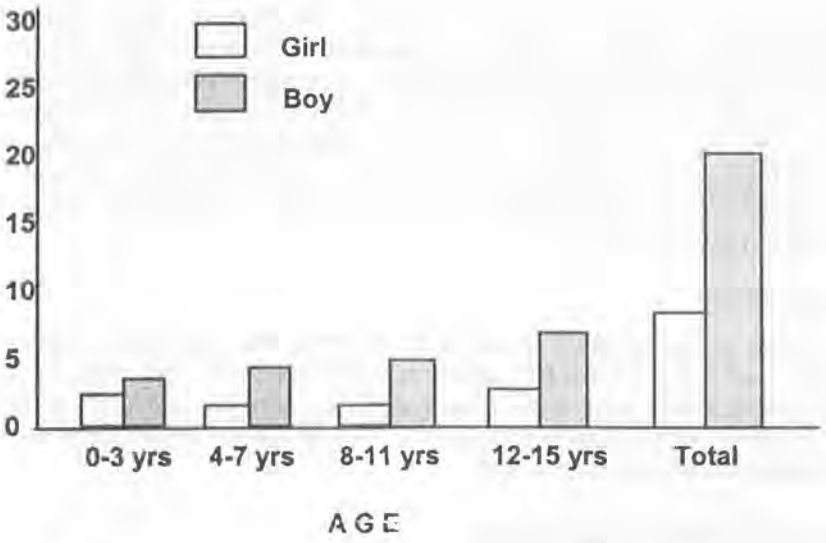

Figure 1. Distribution of primary nephrotic syndrome cases on whom renal biopsy was carried out, according to sex and age.

The youngest age of patient on whom renal biopsy was carried out was 2,5 years, and the oldest, 14.5 years. It was also found that the average age for all cases was 10.5 years, with a median age of 8.5 years. Light microscopy (LM) features of the primary NS cases were very variable as given in Table 1.

Table 1. Number of cases according to the light microscopy (LM) features

Light Microscope Feature	Number	Percentage
o Minimal change (MC)	13	46.4
o Focal segmental glomerulosclerosis (FSGS)	10	35.7
o Membranous glomerulonephritis (MG)	2	7.1
o Mesangial-proliferative glomerulonephritis (MPG)	2	7.1
o Membrano-proliferative glomerulonephritis (MPGN)	1	3.7
Total	28	100.00

The highest frequency was found for minimal changes (i.e. 13 cases, or 46.4%), followed by focal segmental glomerulosclerosis (i.e., 10 cases, or 35.7%). There were only 2 cases (7.1%) or each of membranous glomerulonephritis and mesangial proliferative glomerulonephritis, whereas for membranoproliferative glomerulonephritis there was only 1 case (3.7%). The distribution of cases according to IM features with the various immunoglobulin deposits is shown in Table 2.

Table 2. Distribution of primary nephrotic syndrome cases according to IM features and immunoglobulin deposition

IM Feature	Fluorescence Microscopy Deposit					Total
	IgA	IgG	IgM	C3	Fib	
o Minimal changes	-	-	-	-	-	13
o Focal segmental glomerulosclerosis (FSGS)	-	1	5	3	1	10
o Membranous glomerulonephritis (MG)	-	1	-	-++	-	2
o Mesangial-proliferative glomerulonephritis (MPG)	1	1	2	2	1	2
o Membrano-proliferative glomerulonephritis (MPGN)	-	-	1	1	-	1
Total						28

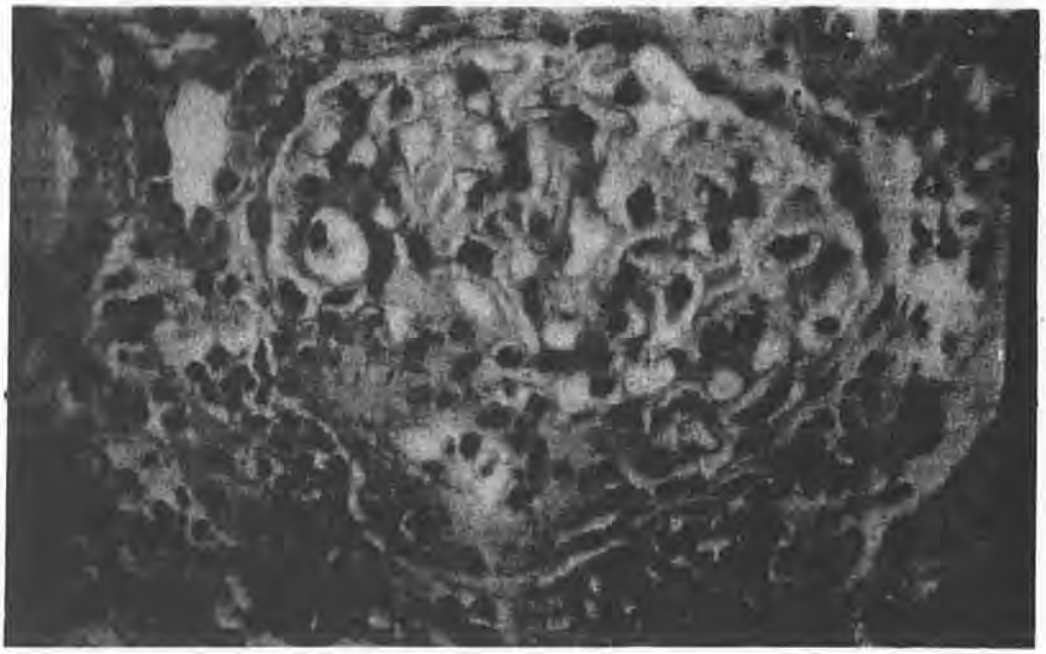

Plate 1. Focal segmental glomerulosclerosis features showing sclerotic glomerulus with segmental expansion (K3 ISN/94).

There were 10/13 (or 76.9%) patients with minimal changes who were sensitive to steroid treatment, which were significantly greater than the insensitive group (i.e. 3/13 or 23.1%) ($p<0.05$), while in the group of patients with focal segmental glomerulosclerosis feature, out of 10 patients there were 7 (or 70%) who were steroid insensitive, and 3 (or 30%) who were steroid sensitive ($p<0.05$).

Based on the other anatomical pathological features, none of the cases was steroid sensitive (Table 3), therefore of all the cases, 13 (or 46,4%) were found to be steroid sensitive and 15 (53.6%) steroid sensitive. In the group of sensitive patients there were 5/13 (38.5%) with hematuria, 2/13 (15.3%) with hypertension, and 2/13 (15.4%) with azothemia, while in the insensitive group there were 6/15 (40%) with hematuria, 5/15 (33.3%) with hypertension, and 5/15 (33.3%) with azothemia (Table 4).

In all cases with minimal changes, there was no occurrence of immunoglobulin deposit. In the 10 cases of focal segmental glomerulosclerosis, various deposits were found as follows: 5 cases with IgM, 3 cases with C3, whereas for IgG and fibrinogen deposits there one cases each. In the two cases of membranous glomerulonephritis, there was only one case with IgG deposit. Of the two cases of mesangialproliferative glomerulonephritis, one case was found having IgA, IgG, IgM, C3 and fibrinogen deposits, whereas in other case, only IgM and C3 deposits were found. In the one membranoproliferative glomerulonephritis case, IgM and C3 deposits were found (Table 2).

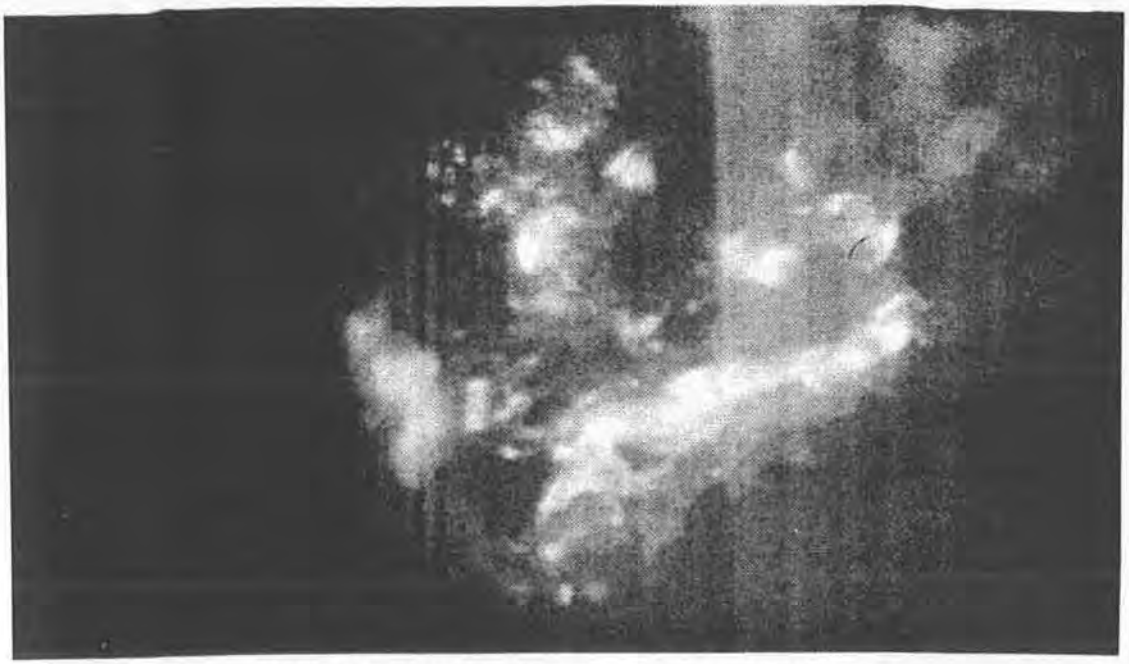

Plate 2. IgA deposit in mesangium and expanding (KS/NS/94).

Table 3. The relationship between various anatomical pathological abnormalities and response to steroid treatment

LM FEATURE	STEROID		TOTAL
	sensitive n=13	insensitive n=15	
○ Minimal changes	10 (76.9%)	3 (23.1%)	13
○ Focal segmental glomerulosclerosis (FSGS)	3 (30%)	7 (70%)	10
○ Membranous glomerulonephritis (MG)	-	2	2
○ Mesangial-proliferative glomerulonephritis (MPG)	-	2	2
○ Membrano-proliferative glomerulonephritis (MPGN)	-	1	1

Table 4. Response to steroid treatment with clinical features (nephritis)

Response	Number	Hematuria	Hypertension	Azothemia
• Sensitive	13	5 (38.5%)	2 (15.4%)	2 (15.4%)
• Insensitive	15	6 (40.0%)	5 (33.3%)	5 (33.3%)

Discussion

Of the 28 nephrotic syndrome (NS) child patients on whom biopsy was carried out, 20/28 (or 71.4%) were males and 8/28 (or 28,6%) were females, giving a male to female ratio of 2.4; 1. This ratio did not differ substantially from the range of 2,0 : 1 to 2,6 : 1 reported by Habib[4] and White et al.[2] The report of Chaw et al.[5] also showed the domination of male patients in NS children. The implementation of routine kidney biopsy on all NS patients has been discontinued,[6,7] due to the fact that primary NS data collection had shown that a larger proportion belong to the minimal changes nephrotic syndrome (MCNS).[8] In this study, the indication for the need of carrying out kidney biopsy were those cases suspected not to be minimal abnormality which were those accompanied by hematuria, hypertension, and patients who did not respond to steroid treatment.

Light Microscope (LM)

Light microscope features in this study were: MCNS 46,4%, FSGS 35.7%, MG 7.1%, MPG 7.1% and MPGN 3.7%, which differ from the ISKDC[6] figures of 76.4% MCNS, 8.6% FSGS, 4,6% PMG and 0.6% MPGN. For the MCNS abnormality the percentages reported by Komiks et al. (i.e., 77%),[9] White et al. (i.e., 82.2%)[10] and Srivasta et al. (i.e. 77%)[11] were substantially higher than whereas that reported by Wirya (i. e., 44.2%)[12] was close to, that obtained in the present study.

Clinical features of FSGS consisted of 60-85% NS with or without microscopic hematuria, 30-60% azothemia, and 24-25% hypertension.[13-15] In this study almost all of the 35.7% FSGS showed nephritic symptoms.

Immunofluorescence Microscope (IM)

In previous IM studies[16,17] no immunoglobulin deposit and complement were found in the MCNS group, and this was also the case in this study. For the GSGS group, IgM deposit was most frequently found spread in the glomerulus depression in the mesangial area or along the glomerulus capillary wall[17,18] and IgG, C3 and fibrinogen

deposits could also be found. Such results were also obtained in this study for several cases. Immunoglobulin deposit and other components could also occur in the other forms (i.e. MG, MPG, MPGN), but in the present study the number of cases only 1-2 for each type.

The number of patients who were steroid sensitive in this study were 76.9% MCNS, which did not differ greatly from the figures previously reported, i.e. 86.1%,[9] 96.9%,[10] and 98% of 134 NS patients treated.[19] The difference between the sensitive and insensitive groups in MCNS was statistically significant ($p<0.05$). In the FSGS group, 70% were insensitive to steroid treatment, which was statistically different from the number of sensitive cases ($p<0.05$).

The occurrences of nephritis in the form of hematuria, hypertension and azothemia were almost similar in the insensitive and sensitive groups. Hence, nephritic symptoms would possibly belong to the non minimal group, and it would be necessary to carry out kidney biopsy to confirm this, whereas non minimal abnormality is generally insensitive to steroid treatment and its prognosis is not so good.[7,20]

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