Diagnosis of glomerulonephritis using light microscopical, immunological and ultrastructural techniques*

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Journal of the Ceylon College of Physicians, 1995, **28**, 46-49

Summary

972 renal biopsies have been studied in the Department of Pathology. Faculty of Medicine, Colombo. 842 were from adults and 130 were from children. 393 patients had nephrotic syndrome and 246 had nephritic syndrome with an unusual course. Other patients had haematuria, proteinuria or renal failure. There were 50 patients who had systemic lupus erythematosus. Renal tissue was obtained using a true cut biopsy needle. Routine histological and immunohistochemical stains were performed using paraffin embedded tissue. Out of 972 biopsies, 801 had sufficient renal tissue. Among 325 adult nephrotics and 108 childhood nephrotics minimal change glomelular disease was seen in 63% and 87% respectively. Mesangial proliferative glomerulonephritis was seen in 20% of adult nephrotics and 7% of childhood nephrotics. Membranous nephropathy was seen only in adult nephrotics (1.5%). Mesangiocapillary glomerulonephritis was seen in 9% of adult nephrotics and 3% of childhood nephrotics. Focal segmental glomerulosclerosis (FSGS) was seen in 5% of adult nephrotics and 3% of childhood nephrotics. 61 adults and 23 children had slowly resolving type of acute diffuse proliferative glomerulonephritis. End stage renal disease was seen in 29 patients. 37 patients had lupus nephritis. 6 patients had interstitial nephritis. Diabetic nephropathy was seen in 4 patients and 2 had amyloidosis of the kidney. In one patient cryoglobulinaemia was diagnosed by renal morphology. Accurate diagnosis of the type of glomerulonephritis is extremely important for the clinician in the management of patients with renal disease.

Introduction

Glomerulonephritis is a disease of both adults and children. However different types of glomerular lesions have different clinical outcomes. Therefore it is very important to know the type of glomerular disease in every patient.

Since the introduction of truecut renal biopsy nearly 50 years ago as an adjunct to the management of patients with renal disease its use has become widespread. The histological pattern of glomerulonephritis is Sri Lankan patients with nephrotic syndrome had been reported by Prof. G Balasubramanium, (et al) in 1983 and by Dr Ramachandran, (et al) in 1971 using histological methods[1,2]. Needle biopsy technique has evolved so that renal tissue can be obtained with ease and safety and the use of immunological and electronmicroscopical techniques has increased the accuracy of diagnosis. Such diagnosis have proved to be of great importance in the clinical management of patients with renal disease in terms of determining therapy and predicting prognosis[3,4,5,6]. By performing light microscopy and immunological stains on true out needle biopsies of the kidney, the pathological changes were studied to arrive at an accurate diagnosis.

The renal pathologist therefore has become and integral part of the clinical team and thus must be aware not only of the pathogenesis and morbid anatomy of renal disease, but also of the clinical nephrologist's approach to diagnosis and clinical management.

Methods

972 renal biopsies were examined. All investigations for renal functions including urine culture, X-Ray of genito urinary tract, ultrasound scan of kidneys, lipid pattern, bleeding time and clotting time were done on every patient. In addition in relevant cases antinuclear factor, anti double and single stranded DNA and LE cells were performed. Renal biopsies were done under local anasthesia using a true cut needle. 2 cores of renal tissue were obtained, one core was fixed in modified Boins' solution for light microscopic examination using haematoxylin and eosin, silver methanamine and periodic acid stains. The other core was fixed in 10% formol saline and immunohistochemical studies[7] using primary rabbit antisera to human IgG, IgM, IgA, C_3 and C_{1q} were performed. In 6 patients .1 cm length of renal tissue was fixed in 4% gluteraldehyde solution and sent to Medical Research Institute for ultrastructural studies. Tissue was processed and semi thin sections were stained with urenyl acetate.

Results

Out of 698 adults the majority (46%) had nephrotic syndrome. 229 (32.8%) had nephritic syndrome with an unusual course. Other forms of renal manifestations are shown in Table 1.

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* *Daphne Attygalle Memorial Oration 1994.*

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Table 1. Clinical Presentation of adult patients n=698

Clinical Presentation	*Number of patients (%)*
Nephrotic syndrome	325 (46.5)
Nephritic syndrome	229 (32.8)
Chronic renal failure	28 (4.8)
Acute renal failure	14 (2)
Haematuria	23 (3.3)
Proteinuria	15 (2.1)
SLE	42 (4.6)
Bartter's	2 (.2)
Transplant rejection	30 (4.3)

Among 103 children, the majority (66%) had nephrotic syndrome with relapses. 16% of children also had nephritic syndrome with an unusually prolonged course. 7.7% were clinically diagnosed as systemic lupus erythematosus. The other renal manifestations in children are shown in Table 2.

Table 2. Clinical picture in children n=103

Clinical features	*Number of patients (%)*
Nephrotic syndrome (relapses)	68 (66)
Nephritic	17 (16.5)
Acute renal failure	4 (3.8)
Chronic renal failure	2 (1.9)
Proteinuria	3 (2.9)
Haematuria	1 (0.9)
SLE	8 (7.7)
Bartters	1 (0.9)

The histological patterns of adult and childhood nephrotic syndrome are shown in Table 3. Minimal change was more common in children than in adults. Membranous nephropathy was not seen among children as shown in Table 3. Mesangio capillary glomerulonephritis and focal segmental glomerulosclerosis (FSGS) were also more commonly seen in adults than in children.

Table 3. Histological patterns of adult and childhood nephrotic syndrome

Type of glomerular disease	*% in adults*	*% in children*
Minimal change	63.5	87.0
Mesangial proliferation	20.5	7.0
Membranous nephropathy	1.5	-
Mesangio capillary GN	9.0	3.0
Focal segmental glomerulosclerosis	5.5	3.0

Immunohistochemical studies revealed IgA immune complexes in 33 patients and 30 of them were adults.

61 adults and 23 children had slowly resolving glomerulonephritis. 38 adults and 2 children had more than 50% epithelial crescents in their renal biopsies. Extensive glomerulosclerosis was seen in 29 patients. 28 of them were adults and one was a child. Out of 28 adults, two had features of chronic pyelonephritis.

37 patients had lupus nephritis. Histologically glomerular lesions were classified according to modified WHO classificattion as shown in Table 4. The majority (72%) had proliferative lesions, either class III or class IV.

Table 4. WHO class of patients with lupus nephritis n=37

WHO class	*Number of patients*
I	-
II	5
III	7
IV	19
V	4
VI	2

4 patients had characteristic lesions of diabetic nephropathy. 2 biopsies showed deposition of amyloid material in the glomerular tufts. Amyloidosis was confirmed after Congo-Red stain.

Discussion

A renal biopsy containing more than 5 glomeruli was considered sufficient for interpretation. Out of 972, only 801 biopsies had sufficient renal tissue.

The commonest renal manifestation both in adults and children was nephrotic syndrome (Table 1 and 2). 63% of adult nephrotics and 87% of childhood nephrotics showed minimal change glomerular disease. Since the introduction of the renal biopsy it has become appreciated that minimal change glomerular disease or lipoid nephrosis is not confined to childhood[8,9]. The onset of childhood nephrotic syndrome was less than 3 years in 70% of children with minimal change and this is in keeping with the international study of kidney disease in children[10].

Mesangial proliferative glomerulonephritis was more common in adults (Table 3) in this series. Histologically expansion of the mesangial matrix and proliferation of mesangial cells were seen. IgA or IgM immune complexes and C_3 were seen in the mesangium.

Membranous glomerulonephropathy is found mainly in adults[11] and it is probably the largest single disease entity in adult patients with nephrotic syndrome. However in this study among nephrotics, only 1.5% had membranous glomerulonephropathy. Histologically with the silver

methanamine stain, the characteristic black "spikes" projecting outwards at right angles to it was seen (Figure 1). In two of these patients electron microscopic studies were performed and subepithelial electron dense immune complexes were found.

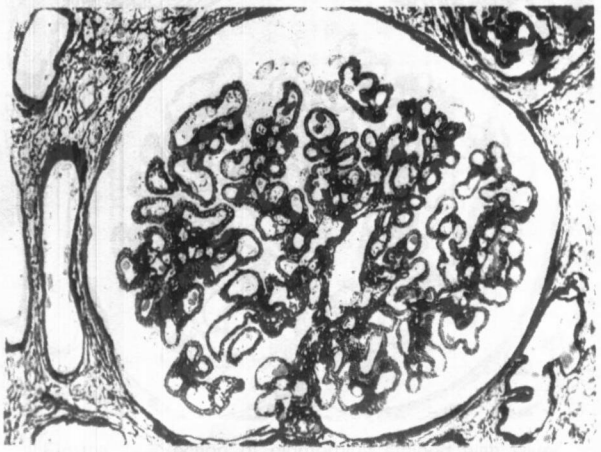

Figure 1. Section of glomerulus stained with silver methanamine showing thickned capillary basement membranes with "spikes".

Membranoproliferative glomerulonephritis has been described most commonly in children and in young adults[12]. In this study this glomerular lesion was found more commonly in adults than in children (Table 3). Out of 51 adults 30 had nephrotic syndrome and 21 had a mixed nephrotic/nephritic picture.

Histologically in the classic form or type 1 disease characteristic thickening and duplication of the basement membrance were seen with silver methanamine stains (Figure 2). Although temporary remission may occur, the prognosis is poor in membranoproliferative glomerulonephritis.

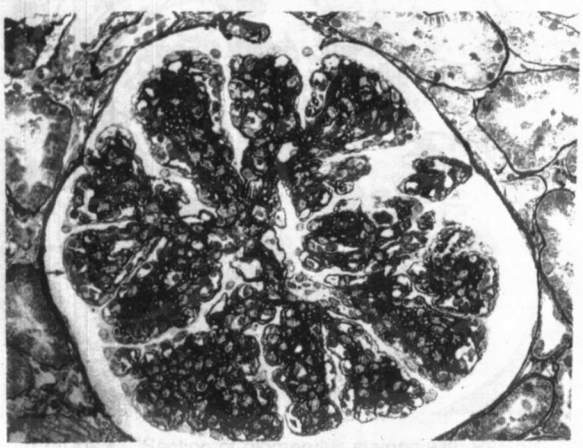

Figure 2. Section of glomerulus stained with PAS/Silver methanamine showing increased tuft cellularity and duplication of the basement membranes.

Focal segmental glomerulo-sclerosis (FSGS) is associated with steroid resistance. The prognosis is poor with progression to renal insufficiency and recurrence of the disease in renal transplant[13]. 26 adults and two children had FSGS. Histologically the lesion consist of glomerulosclerosis involving only a portion of the glomerular tufts (Figure 3).

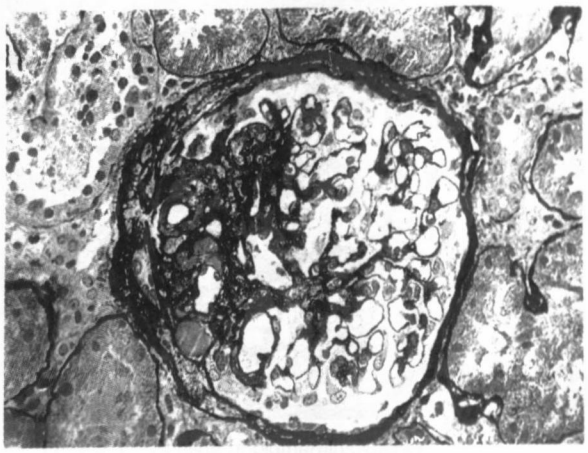

Figure 3. Section of glomerulus stained with PAS/Silver methanamine showing focal segmental glomerulosclerosis.

61 adults and 23 children presented with nephritic syndrome with an unusually prolonged course. Histologically they had mesangial widening and slight increase of tuft cellularity.

These were diagnosed as slowly resolving stage of accute post-streptococcal glomerulonephritis. 38 adults and 2 children had crescentic glomerulonephritis and all had more than 50% epitheial crescents in the renal biopsies.

2 adults had epithelial crescents in almost every glomerulus and died inspite of repeated peritoneal dialysis.

First 10 cases of IgA nephropathy in Sri Lanka had been reported in 1992[14] and the importance of immunological studies in the diagnosis of IgA nephropathy has been stressed[15]. 32 patients had IgA nephropathy. Out of 30 adults the majority (67%) had haematuria. Histologically the predominent pattern was mesangial cell prolifertion with expansion of the matrix.

Out of 698 adults 45% had IgA nephropathy. Higher incidence of 35-40% have been reported from Britain, United States and Canada[16,17]. The low incidence in our country is attributed to many cases being undetected due to the limitations of immunological techniques in the diagnosis of glomerulonephritis.

End stage renal disease was seen in 29 patients and histologically all had extensive glomerulosclerosis and in the majority it was due to chronic glomerulonephritis. Systemic lupus erythematosis (SLE) is a systemic autoimmune disease and clinicopathological correlations have demonstrated a significant relationship between the underlying histopathology of the renal lesion and the subsequent course[18,19]. Out of 37 patients with SLE the majority (70%) had proliferative lesions both focal and diffuse (Table 3).

It has been shown that the survival of patients with proliferative lesions is decreased compared to patients with non-proliferative lesions[18]. Therefore proper evaluation of the renal morphology in patients with lupus nephritis will certainly help in their clinical managemet.

Diabetic nephropathy was diagnosed in 4 patients. The characteristic lesions like Kimmelsteil-Wilson bodies and diffuse ateriosclerosis were seen in all 4.

It was noted that kidney involvement by amyloidosis was rare in Sri Lanka as out of 698 adults, amyloidosis was diagnosed only in two patients. None of the children in this series had renal amyloidosis. Out of the 2 adults, in one amyloidosis was due to multiple myeloma and in the other patient it was due to longstanding rheumatoid arthritis.

Histological diagnosis of glomerulonephritis had been done in the past using a wedge biopsy of the kidney by open surgery. This method has now been replaced by percutaneous true cut biopsy method. This is simpler and much more convenient both for the patient and the clinician. However interpretation of a small core of tissue with limited number of glomeruli obtained by true cut biopsy is a challenge to pathologists compared to the generous piece of tissue obtained by open surgery in the past.

In addition the use of immunological and electromicroscopical techniques has increased the accuracy of diagnosis.

Acknowledgements

I wish to thank Renal Research Fund of the University of Colombo and Hoechst Ceylon Ltd for the grants given to me. I also thank Miss J L Padmani for her technical assistance and Mrs Sudharma Karunaratne for her secretarial assistance.

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