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Original Research Article

Evaluation of renal biopsies in various kidney diseases with reference to staining

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ABSTRACT

Background: Renal diseases of different origin and nature may produce essentially similar disturbances of renal functions and may have clinical similarities and hence there was a need to classify renal diseases more scientifically. The basic approach was to correlate clinical signs and symptoms with histological changes in the tissue, using both simple and special staining techniques so as to reach to a definitive diagnosis.**Material and Methods:** The present study was conducted on renal biopsy referred to pathology department. Criteria for successful biopsy were as follows-Adequate biopsy sample size, correct processing of specimen, informed interpretation and issue of an accurate report.**Results:** A total of 29 renal biopsies were examined. In minimal change disease, only in 4 patients the glomerulus was sclerosed. Membranous glomerulonephritis comprised of the maximum number of cases (9/30). Total of 3 cases of renal biopsies revealed amyloidosis. Focal amyloid deposits with deposits either near the hilum or perivascular areas were found in 33.3% of cases, while extensive amyloid deposits were found in 33.3% of the cases.**Conclusion:** It is necessary to determine both the type of renal disease and the cause of the primary disorder in order to make the diagnosis and various staining techniques play a very helpful role. The likelihood that the biopsy specimen accurately reflects the type and severity of the underlying disease is directly related to both the diffuseness of the disease process and the amount of tissue examined.This is an Open Access (OA) journal, and articles are distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License](https://creativecommons.org/licenses/by-nc-sa/4.0/), which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.For reprints contact: reprint@ipinnovative.com

1. Introduction

Renal diseases of different origin and nature may produce essentially similar disturbances of renal functions and may have clinical similarities and hence there is a need to classify renal diseases more scientifically. The basic approach was to correlate clinical signs and symptoms with biochemical changes in blood and urine along with histological changes in the tissue, using both simple and

special staining and processing techniques so as to reach to a definitive diagnosis. The credit of presenting the first systematic classification goes to Waldherr et al.¹ It was a very large clinico-pathological study based on meticulous gross and microscopic examination of damaged kidneys at autopsies. The renal biopsy subjected to Light microscopy, Immunofluorescence and Electron microscopic studies, remained the mainstay for rational approach to diagnosis, management and assessment of prognosis.

Severe glomerular damage impairs the flow through the peritubular vascular system, conversely tubular destruction

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by increasing intraglomerular pressure which may induce glomerular atrophy.² Thus whatever the origin there is a tendency for all forms of chronic renal disease ultimately to destroy all four components of the kidney, culminating in chronic renal failure.

The various staining techniques- Hematoxylin and eosin (H&E), Periodic Acid Schiff's (PAS), Periodic Acid Schiff's Methanamine (PASM), Congo Red, Methyl Violet, Crystal Violet, Thioflavin-T and other such stains are helpful in the evaluation of renal biopsies in various kidney diseases.

Characterizing chronic kidney disease (CKD) at all stages is an essential part of rational management and the renal biopsy plays a key role in defining the processes involved.³

This study was undertaken because glomerular diseases are the pathologic processes most often encountered in renal biopsy. Specimens and their accurate diagnosis are essential for prognosis and are often a guide to appropriate therapy.

Renal biopsy is an essential procedure in the diagnosis of renal disease, and it is now hard to imagine that one could practice nephrology without knowing pathology.

2. Materials and Methods

This is an observational and retrospective study. Renal biopsies of both male and females of all age groups were considered in this study.

The Inclusion Criteria were renal biopsies of Acute Renal Failure, Nephrotic syndrome, Proteinuria, Haematuria with or without proteinuria, & Systemic Diseases, eg SLE, Henoch schonlein purpura, polyarteritis nodosa, Goodpasture's syndrome, Wegner's granulomatosis and various dysproteinemias.

The Exclusion Criteria were the presence of severe uncontrolled hypertension, Sepsis, known or suspected renal parenchymal infection or haemorrhagic diathesis, solitary ectopic or horseshoe kidney (except in the case of transplanted kidney).

Criteria for a successful biopsy were adequate biopsy sample size, correct processing of specimen, informed interpretation and issue of an accurate report.

Detailed history was collected from the patient's case sheets with special reference to occurrence of oedema, oliguria, hematuria or any other renal abnormality, including hypertension, diabetes or haemorrhagic disease in the family. The following investigation reports were noted: Hb%, TLC, DLC,⁴ Haematocrit values, corrected ESR, bleeding time, coagulation time, prothrombin time, serum proteins, serum albumin, globulin and their A:G ratio, serum cholesterol, blood sugar, blood urea and blood urea nitrogen, alkaline phosphatase and renal function tests like urea, creatinine, Creatinine clearance test, serum sodium, calcium, phosphate, potassium, uric acid, ANA, Anti Ds DNA antibody and C3, C4 fractions, 24 hours urine specific gravity, albumin content and Bence Jones Proteins.

Further, microscopic examination reports for red blood cells, pus cells, casts and crystals. Report of urine culture in suspected cases to correlate with the biopsy report. Sputum examination reports in patients with signs of respiratory lesions and culture for acid fast bacilli, Reports of X-ray (KUB) and Ultrasound were also collected.

All percutaneous biopsies were performed with the guidance of ultrasound. Lower pole of the kidney was selected in most cases and biopsies were performed with a Vim silverman needle. The gross examination of the biopsy was to determine the adequacy of the specimen and to divide the specimen into appropriate portions for subsequent processing. The overall length, colour and consistency of the tissue specimen was noted in particular, the area of viable cortex was identified and delineated from the medulla; which was generally more pale. Areas of infarction, necrosis- pyogenic inflammation were also pale, but were highlighted by a hyperemic border.

After fixation the tissue was dehydrated and embedded in paraffin wax because it was automated and permitted the use of the greatest variety of special stains. Normally seven slides were prepared with 2 μ m thick sections.⁵ The first, fourth and seventh slides were stained with Haematoxylin & Eosin, the second and fifth slides stained with Periodic Acid Schiff (PAS) and third and sixth slides with Periodic acid methenamine silver (PAMS) staining Additional stains like Congo red, Methyl violet, Crystal violet and Thioflavin T were used to demonstrate amyloid.

2.1. Statistical analysis

Descriptive analysis of the study was performed by using excel sheet. Percentage distribution was calculated.

3. Results

A total of 29 cases of renal biopsies were examined out of which 20 biopsies belonged to males and 9 biopsies belonged to females. Biopsies were of Acute renal failure, nephrotic syndrome with or without hematuria and kidney transplant (Table 1).

Table 1: Type of renal disease and percentage

Type of renal disease	No. of Patients (%)
Minimal change disease	7(24%)
Membranous	9(31%)
Amyloidosis	3(10%)
Focal segmental glomerulosclerosis	4(13%)
Mesangioproliferative glomerulosclerosis	2(6%)
Systemic lupus erythematosus	1(3%)
Transplant rejection	1(3%)

Minimal change disease comprised of 7 patients including 6 male and only 1 female within an age group of

15 - 55 years. In most of the patients (86%) the glomerulus was neatly showing no apparent change. Only in 4 patients the glomerulus was sclerosed (Figure 1).

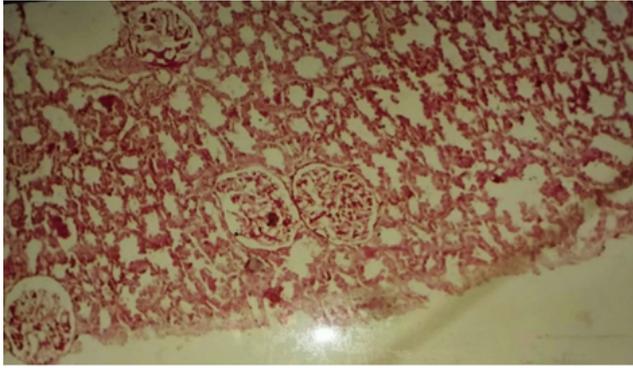


Fig. 1: Minimal change disease -both glomeruli show normal cellularity

In our study mesangium showed normal cellularity in 4 cases while increased cellularity in 3 cases. Out of these 2 biopsies showed mild segmental hypercellularity while 1 biopsy showed diffuse mesangial hypercellularity. Tubules were unremarkable in most of the cases and in only one patient tubular atrophy was observed. Vasculature and interstitium showed no significant findings. In 1 case interstitium showed mononuclear inflammatory cells in 3 (43%) of patients.

Two cases of membranoproliferative glomerulonephritis (MPGN) were identified showing 27-28 glomeruli, several tubules and vessels. All glomeruli were enlarged with a diffuse increase in glomerular tuft cellularity. The hypercellularity was global, creating lobulation. There is thickening of glomerular basement membranes along with mesangial proliferation leading to lumina reduction of glomerular capillaries. Tubules and vessels were unremarkable and interstitium showed focal inflammatory infiltrate (Figure 2).

Focal segmental glomerulosclerosis is a clinicopathologic entity characterized morphologically by segmental areas of sclerosis in some of the glomeruli (Fogo AB).⁶ We studied 4 cases of renal biopsies containing 10 - 12 glomeruli on average. Almost 30% of the glomeruli showed segmental lesions while the rest of the glomeruli were unscarred with either enlarged or normal glomeruli. As regards the site of lesion, it was at the origin of proximal convoluted tubule (tip lesion) and hilum in 25% cases each and indeterminate in 50% of cases. Foam cells were identified in 25% of cases. Glomerular hyalinosis was present in 50% cases. Tubules showed atrophy in 50% of cases while interstitium showed fibrosis in 50% of biopsies (Figure 3).

A total of 3 cases of renal biopsies revealed amyloidosis. Focal amyloid deposits with deposits either near the hilum

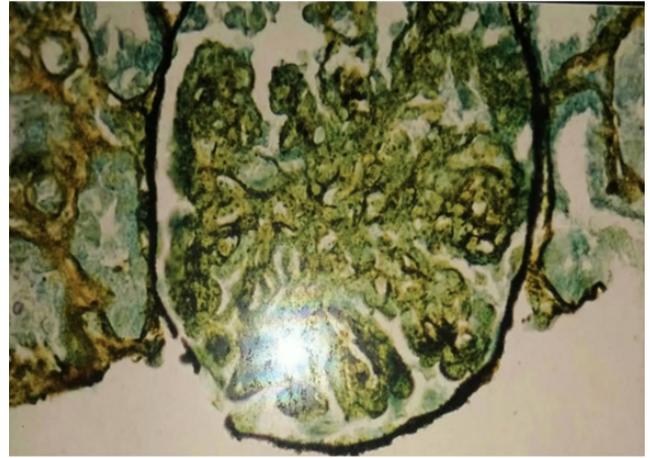


Fig. 2: Membranoproliferative Glomerulonephritis - Lobule formation with inflammation

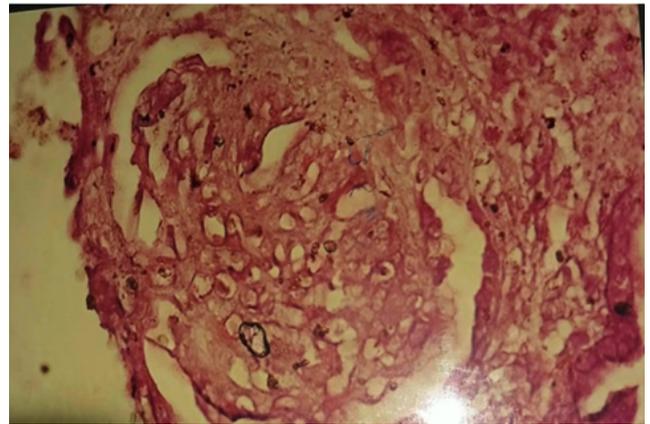


Fig. 3: Focal Segmental Glomerulosclerosis: Focus of segmental sclerosis observed

or perivascular areas were found in 33.3% of cases while extensive amyloid deposits were found in 33.3% of the cases. In one patient the deposit was so extensive that glomerulus underwent complete obsolescence. Although we did not find any abnormality in basement membranes in our study (Figure 4).

In this study there was a single case of hyperacute rejection occurring immediately. The patient developed anuria and kidneys showed no perfusion. Serial biopsies were taken on day 1, 5 and 8. Light microscopy showed on Day 1: Well preserved renal architecture. Glomerular capillaries were dilated and lumen was plugged with amorphous masses of platelets. Tubular epithelium showed swelling, focal areas of necrosis, ruptured basement membranes and lumina occluded by haemorrhagic casts. Interstitium was edematous and showed sparse inflammatory infiltrate. Day V - showed disruption of the normal kidney architecture. Glomerular capillaries were

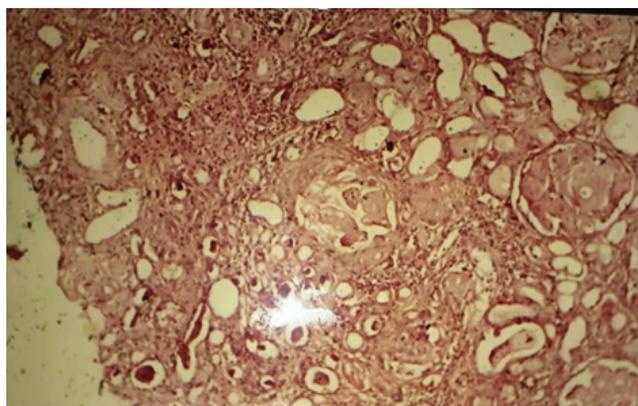


Fig. 4: Amyloidosis: Focal amyloid deposits

intensely congested. There was endothelial damage of small arteries, arterioles, glomeruli and peritubular capillaries and capillaries filled with sludged (compacted) red cells and fibrin. Neutrophils began to infiltrate in glomerular capillaries. Day VIII -Section showed features of renal infarction with only ghosts of normal structures identified with intense neutrophil margination so that they form chain like figures.

Mesangioproliferative glomerulonephritis has been defined by the WHO committee as a uniform increase in mesangial cells. (4 or more in number) in nearly all glomeruli.⁷ In this study two renal biopsies presented with features of MESGN. Glomerulus revealed mesangial proliferation in all the cases. However, capillary was patent in all cases. Basement membrane was normal in 50% cases while it thickened in the remainder. Baldwin et al⁸ in a group of 603 patients demonstrated mesangial hypercellularity and graded it from minimal to severe with two intermediate grades. 75% fell into two minor grades and only 3% was the hypercellularity graded as severe. No significant changes were observed in the tubules, interstitium and vasculature (Figure 5).

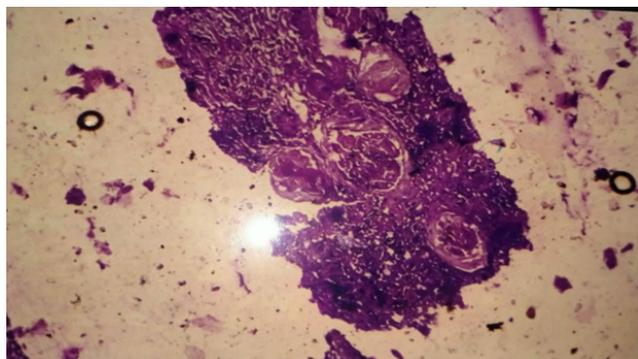


Fig. 5: Mesangioproliferative Glomerulonephritis - Mesangial hypercellularity

Only a single case of SLE was diagnosed in our series of renal biopsies. The patient was a 36 years old female presented with nephrotic syndrome, haematuria, hypertension along with joint pains and malar rash. Amongst the serologic tests Antinuclear antibodies were positive and C3 and C4 complement levels were reduced. Light Microscopy revealed diffuse and global lesions (Figure 6).



Fig. 6: Systemic lupus erythematosus: Silver methenamine stain

4. Discussion

This study was undertaken with the objective that various staining techniques help as an adjunct in the evaluation of various kidney diseases. The various staining techniques clearly reveal the structure of glomerulus, tubules and the vessels and their deposits under light microscope to differentiate various kidney diseases

White et al⁹ suggested that focal hyalinized/obsolete glomerulus may be present in a renal biopsy suggestive of minimal change disease, especially if they are not associated with interstitial fibrosis and tubular atrophy (National Kidney Foundation).¹⁰

Walker PD et al¹¹ stated that in most patients, mesangial cellularity is normal but two subgroups with more numerous mesangial cells have been described: mild, usually segmental hypercellularity and diffuse mesangial hypercellularity proliferation.¹²

Membranous glomerulonephritis comprised of the maximum number of cases (9/30) of our study. Most of the patients were males (6/9,70%) and were in the age group of 35 - 45 yrs. In our study most of the glomeruli were enlarged in size (78%), one glomerulus was normal while one showed crescent or scar formation. The earliest change is mottling of the GBM seen in face sections stained with methenamine silver - PAS. The present study showed uniformly thick capillary basement membrane in 3 cases (33%) irregular in 3 cases (33%), spikes in 2 cases (22%) and domes in 1 case (11%). Tagushi and Bohle¹³ found single, scattered barely visible spikes in 21% of

472 cases of MGN and in 25 cases the changes were so minimal that the diagnosis could only be established after immunohistology or electron microscopy. Mesangium showed normal cellularity in 6 and increased cellularity in 3 cases. Tubules were unremarkable in 78% of cases while 2 biopsies showed atrophied tubules. Most of the vasculature showed arteriolar thickening (89.9%) and infiltration with chronic inflammatory infiltrate (55.6%).

Caoli EM et al¹⁴ have described sequential biopsies from 33 patients with MPGN (type I and II). Morphologic changes in the tubules and interstitium generally reflect the changes noted in the glomeruli.

Wehrmann et al¹⁵ in a study of 250 patients found that patients with normal interstitium had a 5 year survival rate of 98% and a 10 year survival rate of 83%.

Bell ET¹⁶ stated that amyloid extends from the mesangium into the subendothelium in most cases. Watanabe et al¹⁷ reported a case with an apparent reaction to glomerular amyloid with macrophages, giant cells and associated crescents. Vasculature was unremarkable in 33.3% of cases, showed focal deposits in the walls in another 33.3% of cases while lumen obliteration was seen in the rest of the cases. Bohle et al¹⁸ found amyloid in interlobular arteries or arterioles in 97 of 122 renal biopsies. Interstitium and Tubules-were unremarkable in 33.3% of cases but showed deposits in 66.6% of cases. Ginzburg S et al¹⁹ reported similar interstitial involvement.

Study by Campbell et al²⁰ has shown that 41 patients out of 44 patients with diffuse proliferative lupus nephritis and nephrotic proteinuria in which 26 patients had as an initial findings and in 15 during the course of the renal disease.

5. Conclusion

We conclude that light microscopic evaluation of renal biopsies using special staining techniques has helped much to understand the histological diagnosis of kidney diseases because renal disturbances of different pathogenesis may end in stages which are morphologically similar and not easy to differentiate.

Glomerular diseases are the pathologic processes most often encountered in renal biopsy and their accurate diagnosis is essential for prognosis and a guide to appropriate therapy. The histological diagnosis included the maximum number of cases of membranous glomerulonephritis with minimal change disease at the second position and focal segmental glomerulonephritis at the third place.(S Sethi et al)²¹ Amyloidosis, mesangioproliferative glomerulonephritis constituted about 6% each while membranoproliferative glomerulonephritis, SLE and transplant rejection 3% cases each. It should be emphasized that although renal biopsy was instrumental in investigations regarding the pathogenesis and treatment of renal diseases, there has not been a consensus about when to perform renal biopsy in clinical practice.

Identification of the type of renal disease by biopsy is not synonymous with identifying the patient's diagnosis. In a patient with renal disease that is secondary to another disease, it is necessary to determine both the type of renal disease and the cause of the primary disorder in order to make the diagnosis. It is also important to appreciate the relationship of structural alterations identified in the core of renal tissue removed during the biopsy to alterations within the entire kidney. The likelihood that the biopsy specimen accurately reflects the type and severity of the underlying disease is directly related to both the diffuseness of the disease process and the amount of tissue examined.

Finally the validity of the extent of a damage as an index of severity of renal disease is correlated with the severity of functional alterations and further with the prognosis.

So it is concluded that light microscopy, immunofluorescence and electron microscopic findings should be integrated to derive a histologic diagnosis that is descriptive of the disease process. The histologic diagnosis is then co-related to the clinical findings to give a clinicopathologic diagnosis that can be used to plan a course of therapy and establish the prognosis.

6. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

7. Source of Funding

None.

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