"EVALUATION OF RENAL BIOPSIES: A SINGLE INSTITUTIONAL EXPERIENCE."

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ABSRTACT:

Aims & Objectives:Renal biopsy is an essential tool and is the gold standardmethod for the diagnosis of glomerular diseases. It is useful for identifying the specific diagnosis, assessing the level of disease activity, and for allowing specific decisions about treatment to be made.In this study,we tried to examine and interpret renal biopsies using lightmicroscopy, special stains and immunofluorescence, to study the histological pattern of renal diseases on biopsies and correlate clinically

Material and Methods: A prospective andreterospectiveanalysis of hundred renal biopsies was done.A minimum of two cores required,one core for Direct immunofluorescence(DIF) in normal saline,another in formalin forLight microscopy(LM) .DIF staining were done withantibodies against Immunoglobulins A, G, M, and C3c, and C1q.In light Microscopy, biopsies were stained routinely with Hematoxylin and Eosin(H&E) and Special stains - Periodic acid schiff(PAS), Masson Trichrome(MT), Silver methenamine(SM)

Results:The study included 100 cases.The age distribution among the lesions ranged from 13 years to 72 years.55 cases were males and 45 were females.These included various glomerular and nonglomerulardiseases.Membranoproliferative glomerulonephritis, (*n* = 17)and tubulointerstitial disease,(n=17) being the commonest followed by membranous glomerulopathy (*n* = 16);diabetic nephropathy (n=11):post infectious glomerulonephritis(n=10); HTN nephropathy(n=8); IGA nephropathy(n=6); Focal segmental glomerulonephritis(n=5); Minimal change disease(n=5);crescentic Glomerulonephritis(*n* = 3); Lupus nephritis(n=2).

Conclusion:1. Renal biopsy is an essential tool in the diagnosis of glomerular diseases.
2. Immunofluorescence plays an important role in isolating and identifying immune deposits in glomerular diseases

Key Words:Renal biosy, Light Microscopy,Immunofluroscence.

Introduction:

Renal diseases are responsible for a great deal of morbidity and mortality and put a great burden on the health care system. It has varied clinical manifestation and poses a challenge to the clinicians. The reported prevalence of chronic kidney disease (CKD) in different regions ranges from 1% to 13%, and recently, data from the International Society of Nephrology's Kidney Disease Data Center Study reported a prevalence of 17%.[1]

The etiology of CKD varies considerably throughout India. Parts of the states of Andhra Pradesh, Odisha, and Goa have high levels of CKD of unknown etiology , which is a chronic interstitial nephropathy with insidious onset and slow progression.[2]

Renal biopsy is an essential tool and is the gold standard method for the diagnosis of glomerular diseases.[3]It is useful for identifying the specific diagnosis, assessing the level of disease activity, and for allowing specific decisions about treatment to be made. The ability to differentiate between immune complex (IC) mediated glomerulonephritis (ICGN) and non-ICGN is crucial for appropriate therapeutic decisions.[4]Therefore, evaluation of glomerular ICs is an essential component of the pathology of renal biopsy specimens

The first systematic classification of renal diseases was given by Waldherret al.[5]

Routinely the kidney biopsy is examined by

• Light microscopy (LM) for morphologic changes using various stains such as Hematoxylin and eosin (H and E), Periodic acid-Schiff (PAS),Silver methenamine (SM), and Masson's Trichrome (MT)

• Direct immunofluorescence (DIF) for evaluation of immune deposits

• Electron microscopy for ultrastructural evaluation.

The method for Imunofluorescence (IF) labeling was developed by Coons and Kaplan in 1950s.[4] IF on frozen sections has been the gold standard for immunological evaluation of renal biopsy specimens.[6]The IF method is relatively simple, highly sensitive, and very specific. Diseases such as IgA nephropathy ,C1q nephropathy and C3 glomerulopathy cannot be diagnosed without IF. Direct immunofluorescence on fresh frozen tissue is the most widely used IF technique without which it leads to incomplete diagnosis and suboptimal patient management.[7]

Aims and Objectives of the study:

1. To examine and interpret renal biopsies using light microscopy, special stains and immunofluorescence.
2. To study the histological pattern of renal diseases on biopsies.

Materials and methods:

A prospective and reterospective analysis of all the renal biopsies done and sent to Dept of Pathology over a period of two years which included 100 biopsies. Inadequate and renal allograft biopsies were excluded from the study.

All the biopsies were performed by a well experienced nephrologist or radiologist using an automatic triggered biopsy gun .Clinical presentation, blood pressure, blood sugar, blood urea, serum creatinine, urine analysis were noted in all cases.

A minimum of two cores required, one core for DIF in normal saline, another in formalin for LM.DIF staining were done with antibodies against Immunoglobulins (Ig) A, G, M, C3c, and C1q.In light Microscopy, biopsies were stained routinely with Hematoxylin and Eosin and Special stains – PAS,SM,MT .

Immunofluorescence method: Fresh and unfixed renal biopsy specimens were received in normal saline or Michels media for immunofluorescence.

Multiple 4-5 μm thick sections of the biopsy tissue were prepared on the cryostat and air dried for at least 60 minutes at room temperature. The sections were covered with Phosphate Buffered Saline(PBS). Respective sections were overlayed with 25 μl of appropriate fluorescein isothiocynate (FITC) - conjugated antisera (antisera against human IgM, IgG, IgA and C3 were used). The FITC- conjugated antisera against IgG, IgA, IgM and C3 of Dako Company were used. The sections were then incubate in moist chamber at room temperature for 30 minutes and then washed in PBS at room temperature in a dark place for 10 minutes. One to two drops of mounting medium were placed on the slides and slides were examined under the fluorescent microscope.

The positivity and staining pattern of individual antihuman antibodies were noted. Grading of the intensity in immunofluorescence was determined semiquantitatively using a scale from 0 to 4+, in a manner similar to guidelines established by the Centers for Disease Control and Prevention, Atlanta, Georgia for indirect IF: 0, 1+, 2+, 3+, and 4+ for nil, mild, moderate, moderately severe, and severe staining.[8]

The basic morphology was studied on light microscopy and later the immunodeposits of antibodies were categozied by the pattern of deposits and intensity of staining with IF

Results:

The study included 100 cases, of which Membranoproliferative glomerulonephritis and tubulointersitial nephritis were the most common followed by membranous glomerulonephritis.

TABLE 1: Incidence of lesions

Diagnosis	Total of cases(n=100)
Membranoproliferative glomerulonephritis(MPGN)	17
Tubulointerstitial nephritis (TIN)	17
Membranous glomerulonephritis(MGN)	16
Diabetic nephropathy	11
Post infectious glomerulonephritis(PIGN)	10
Hypertensive(HTN) nephropathy	8
IgA nephropathy	6
Focal segmental glomerulonephritis(FSGS)	5
Minimal change disease(MCD)	5
Crescentric glomerulonephritis	3
Lupus nephritis	2

The age distribution among the lesions ranged from 13 years to 72 years. Mean ± Standard Deviation of age is (41.22 ± 15.68).

TABLE 2: Age distribution of lesions

AGE	n
10-20	12
21-30	16
31-40	24
41-50	17
51-60	18
61-70	11
71-80	2
Total	**100**

TABLE 3: Gender distributions among glomerulopathesis.

Among 100 cases of gomerlupathesis,55 cases were males and 45 were females.

Gender	No. of cases(n)
Male	55
Female	45
Total	**100**

TABLE 4:Immunofluorescence findings:

Diagnosis on IF	FREQUENCY	PERCENTAGE
No Immune Deposits	30	30%
Membranoproliferative glomerulonephritis	17	17%
Membranous glomerulonephritis	14	14%
Post infectious glomerulonephritis	10	10%
Diabetic nephropathy	9	9%
IGA nephropathy	6	6%
HTN nephropathy	5	5%
Focal segmental glomerulonephritis	4	4%
Crescentric glomerulonephritis	3	3%
Lupus nephritis	2	2%
TOTAL	**100**	**100**

In the present study immunofluorescence findings showed no immune depositis in majority of case accounting for thirty cases which includes Tubulointerstitialnephritis(n=17),Minimal change disease(n=5), HTN nephropathy (n=3) ,Membranous glomerulonephritis (n=2) ,Diabetic nephropathy (n=2) ,Focal segmental glomerulonephritis (n=1)

Discussion:

Kidney disease with its high prevalence, morbidity and mortality, is an important public health problem. Prevention and early detection of CKD mandate involvement of physicians, nephrologist and pathologist at all levels. Most patients with CKD can be managed by their nephrologist with timely referrals for renal biopsy examination[9] Hence,

histopathological examination is a must for final definitive diagnosis along with the use of other methods like special stains and Immunofluorescence.

In the present study,100 cases of various glomerulopathies and interstitial nephritis were analyzed over a period of two years at our institute .

After excluding the inadequate samples, 100 eligible cases were assessed both by Light microscopy and IF.

The age distribution among the lesions ranged from 13 years to 72 years. Mean age is 41.22 years. Majority of cases were between 31-40 years (n=24) followed by 51-60(n=18) and least no of cases were noted in age group of 71-80 years(n=2).[table-4]

In our study, there was male predominance .Among 100 cases of gomerolupathesis,55 cases were males, 45 were females. This observation is similar to various Indian and international studies.[9-16]

These included various glomerular and nonglomerular diseases. Membranoproliferative glomerulonephritis, (*n* = 17)and tubulointerstitial disease,(n=17) being the commonest followed by membranous glomerulopathy (*n* = 16);diabetic nephropathy (n=11):post infectious glomerulonephritis(n=10); HTN nephropathy(n=8); IGA nephropathy(n=6); Focal segmental glomerulonephritis(n=5); Minimal change disease(n=5);crescentic Glomerulonephritis(*n* = 3); Lupus nephritis(n=2).[table-3].

Membranoproliferativeglomerulonephritis(**n**=17) is the most common subtype of glomerulopathy detected in our study.

MCD was the most common lesion in studies by Varun et al [17] and Das et al [10], whereas FSGS was the commonest histology in studies done by Balakrishnan et al [18] and Mubarak et al[16.] In a registry study from the Far-east, Chang et al [17] reported that the most common primary GN was IgAN (28.3%), which was followed by MCD (15.5%). In a study from Brazil, FSGS (24.6%) was the most frequent primary glomerular disease, followed closely
by MN (20.7%) and IgAN (20.1%).[20]

Histopathology of MPGN showed enlarged glomeruli with capillary wall thickened and prominent mesangial proliferation on light microscopy and tram track pattern seen in PAS staining in few of the cases.Granular deposits with mainly IgG and C3c along the capillary walls and mesangium which showed intensity of 1+ to 4+ on immunofluorescence.(Figure1)

Caoli EM et al[21] 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

Membranous glomerulonephritis is thesecond commonest glomerular lesion(n=14)in our study. Majority showed histologically capillary wall thickening which corresponded with immunofluorscence finding of granular deposits on capillary wall mainly with IgG and C3c with intensity of 1+ to 4+..(Figure2)

On microscopy, four stages of the disease process have been identified. In Stage I, light microscopyshows no GBM thickening. EM shows sub-epithelial deposits focally.

In stages II and III, light microscopy shows the thickening of the GBM.PAS and SM stain shows characteristic spike-like formations.[22]

In stage IV, Immune complex deposits are incorporated in the GBM. At this stage, the thickness of GBM is more appreciated than spikes.

The sub-epithelial deposits on electron microscopy (EM) are considered to be pathognomonic for MN.[20] Immune mediated Glomerular basement membrane results in proteinuria due to podocyte structural damage .[23]

Post infectious glomerulonephritis (n=10) on light microscopy showed mesangial proliferation and accentuation of the glomeruli with leukocytic infiltration. On immunofluorescence revealed granular deposits with IgG and C3c mainly along the capillary wall with 1+ to 4+ intensity.

Diabetic nephropathy (n=9) on light microscopy showed mainly diifuseglomerulosclerosis with diffuse linear depositis on glomerular capillary wall, tubules with IgG mainly with 1+ to 3+ intensity on immunofluorescence.

Ig A nephropathy (n=6) showed increased mesangial matrix and thickened basement membrane on light microscopy with IgA deposits along the mesangium with 2+ to 4+ intensity.

HTN nephropathy(n=5) showed increased glomerulobasement membrane with vessels sclerosis on light microscopy with IgG and c3c deposits on arterioles with 2+ to 3+ intensity .

Focal segmental glomerulonephritis (n=4) showed segmental sclerosis with increased mesangial matrix with mild mesangialhypercellularity on light microscopy with mainly IgM and C3c deposits with 1+ to 3+ intensity.

Crescentric Glomerulonephritis (n=3) showed crescentric formation on light miscroscopy with IgG and C3c deposits mainly under immunofluorescence. Pauci immune crescentric showed 2+ intensity deposits with IgG and C3c.Another was given as immune complex crescentric glomerulonephritis showed IgG and IgM deposits with 3+ and 2+ intensity respectively.

Lupus nephritis(n=2) was diagnosed as Diffuse lupus nephritis(grade 4 a) on light microscopy due the presence of >50% glomeruli showing crescents and increase mesangium,thickened basement membrane with capillary wire loop lesions.

On immunofluorescence it showed full house positivity with varying intensity grade.

LN was the most common secondary glomerular disease in several studies.16,20,23-27

Tubulo interstitial nephritis was seen in 17 % cases. Varun et al reported tubulointerstitial nephritis in 17.5% of cases and Mubarak et al in 11.6% of cases. Progressive glomerular injury is accompanied by chronic injuries to other renal structures, typically manifest as tubulointerstitial fibrosis. Tubulointerstitial nephritis(n=17) showed no involvement of glomeruli and vessels with thickened basement membrane of tubules with neutrophils infiltrate under light microscopy.Immunofluorescence was negative for IgG,IgA,IgM,C3c,C1q.

Table 5: Comparison of data from different previous Indian studies with our study.

v	Present study	Das et al [10]	Balkrishnan et al [18]	Varunetal[17]
Number of subjects	100	1849	5016	924
M:F	1.2:1	1.5:1		1.5:1
Membranoproliferative glomerulonephritis	17%	3.9%	2.9%	2.4%
Tubulointerstitial	17%	3.7%	2.7%	17.5%

nephritis				
Membranous glomerulonephritis	16%	15.1%	10.8%	11%
Diabetic nephropathy	11%	1.2%	2.8%	2.6%
Post infectious glomerulonephritis	10%	4.7%	13.5%	6.3%
HTN nephropathy	8%	-	-	-
IGA nephropathy	6%	4.4%	8.4%	14.3%
Focal segmental glomerulonephritis	5%	10.5%	16.8%	10.6%
Minimal change disease	5%	15.1%	10.8%	15.4%
Crescentric glomerulonephritis	3%	4.5%	-	-
Lupus nephritis	2%	14.6%	6.9%	4.9%

Other studies included amyloidosis, Thrombotic microangiopathy, Chronic sclerosing glomerulonephritis,C1q nephropathy, Focal necrotizing glomerulonephritis, Acute tubular necrosis, Thrombotic microangiopathy in their results. They are not encountered in our study.

In this study, the results of staining of individual antibodies i.e. IgG, IgA, IgM ,C3c and C1q were recorded as positive or negative for IF

On individual assessment all of the deposits showed an intensity of 1+ to 4+ on Immunofluorescence.

Membranoproliferative glomerulonephritis (n=17) being the commonest followed by membranous glomerulonephritis(n=14);post infectious glomerulonephritis (n=10),diabetic nephropathy (n=9);IgA nephropathy(n=6);HTN nephropathy (n=5);focal segmental glomerulonephritis(n=4);crescentric glomerulonephritis (n=3);lupus nephritis(n=2).).[table-4].

In the present study immunofluorescence findings showed no immune depositis in majority of case accounting for 30 cases which includes

Tubulointerstitialnephritis(n=17) showed no involvement of glomeruli and vessels with thickened basement membrane of tubules with neutrophils infiltrate under light microscopy. Under immunofluorescence was negative for IgG,IgA,IgM,C3c,C1q.

Minimal change disease(n=5) showed no changes in light microscopy and no immune deposits in immunofluorescence.

HTN nephropathy (n=3) showed normal glomeruli with mild thickening of GBM but no immunodeposits under immunofluorescence.

Membranous glomerulonephritis (n=2) showed mild GBM thickening and capillary wall thickening at places but no immunodeposits.

Diabetic nephropathy (n=2) showed glomeruli sclerosis with thickened GBM but no immunodeposits.

Focal segmental glomerulonephritis (n=1) showed glomeruli sclerosed and few glomeruli showed increase GBM and increase mesangial matrix but no immunodeposits in immunofluoscence.

Summary & conclusion:

1. Renal biopsy is an essential tool in the diagnosis of glomerular diseases.
2. Immunofluorescence plays an important role in isolating and identifying immune deposits in glomerular diseases

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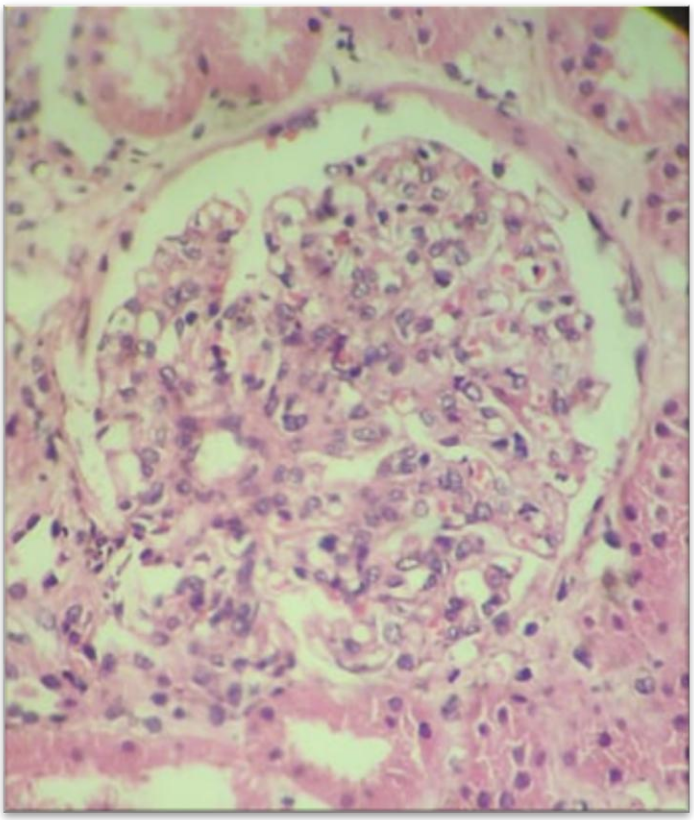
Figure1:Membranoproliferative glomerulonephritis



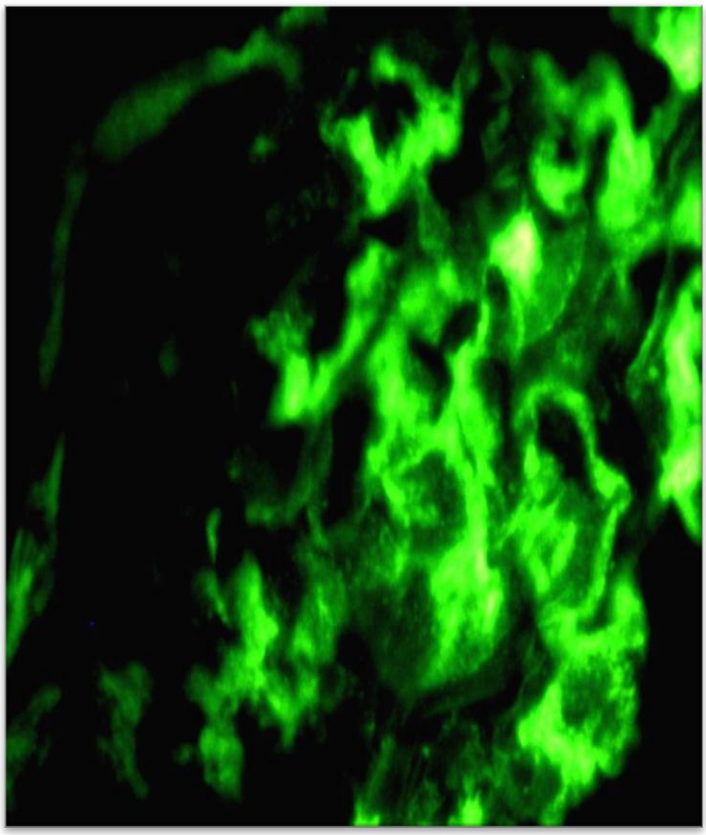


Figure 1a:Glomerrulus showing endocapillary proliferation, thickening of basement membrane and increase mesangial cellularity.(H & E 40X)
Figure 1 b:IF showing 3+ coarse granular IgGdeposts in GBM and mesangium.

Figure 2: Membranous Glomerulonephritis

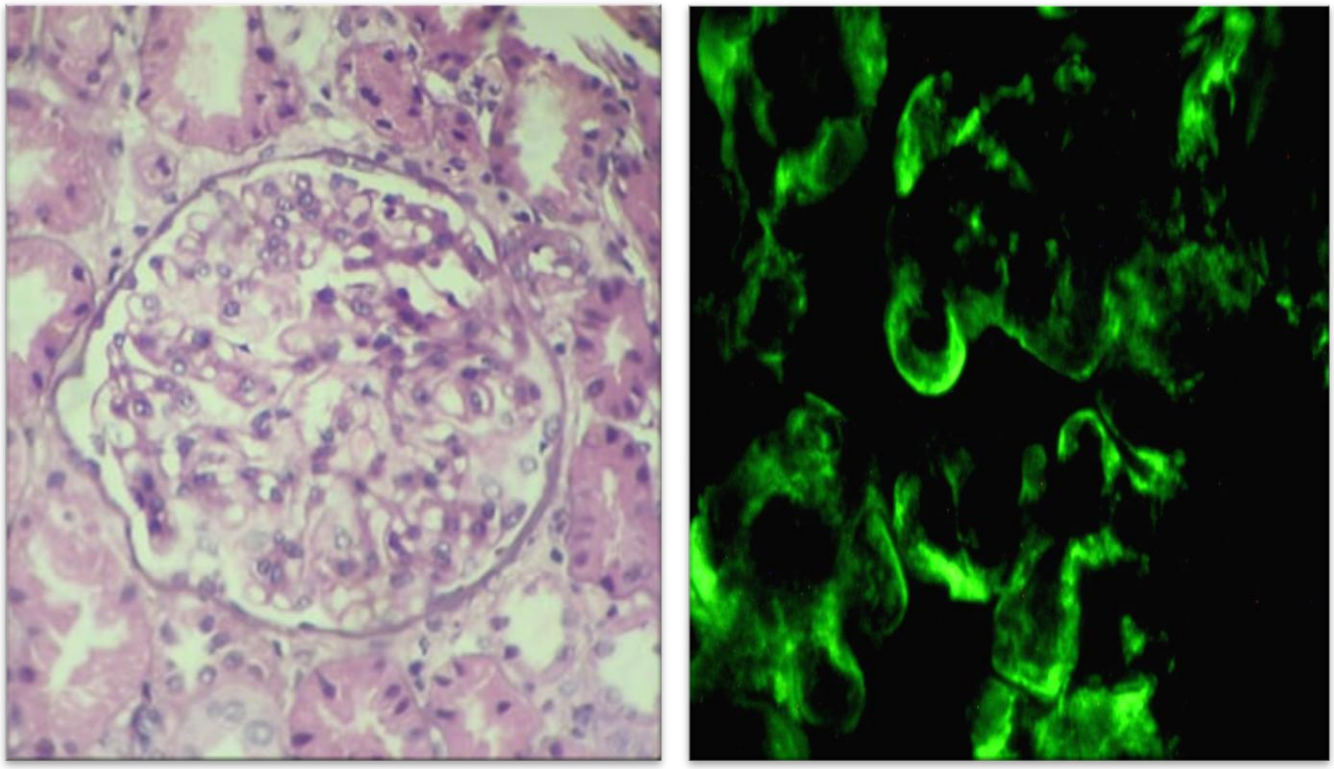


Figure 2a: Glomerulus showing endocapillary proliferation and thickened basement membraneand,mild increase in mesangium.(H&E 40X)

Figure 2b:IF shows granular deposits IgG with 2+ intensity