Original Article

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Morphological Spectrum of Lesions in Renal Biopsies with Diagnostic Role of Immunofluorescence: A Study in a Tertiary Care Centre

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ABSTRACT

Background: Renal diseases are common causes of morbidity in clinical practice and their incidence is on rise. Glomerulonephritis constitutes nearly 60% of all non-surgical renal diseases and accounts for a substantial number of cases of end stage renal disease.

Objectives: This study was done to analyse the histomorphology of renal diseases. Specific immunofluorescence patterns were also studied as an aid to diagnose various lesions.

Materials and Methods: This study was done for a period of six months between January 2017 and June 2017. A total of 30 renal biopsies were received in the Department of Pathology, Coimbatore Medical College, Coimbatore. The tissues were subjected to light microscopic examination and immunofluorescence studies.

Results: Among the total 30 renal biopsies, the most common age group affected was between 31 years and 40 years. The most common age group affected was between 31 years to 40 years. Females (51.72%) were slightly more affected than males (48.27%). Out of 30 cases, 23 (79.31%) showed primary glomerular lesions, 5 (17.24%) showed secondary glomerular lesion and 2 (3.45%) showed tubulointerstitial nephritis. Diffuse proliferative glomerulonephritis was the most common primary glomerular lesion with a total of 6 out of 30 cases (22.41%). Lupus nephritis was the most common secondary glomerular lesion with a total of 4 out of 30 cases (12.07%). Immunofluorescence studies showed positivity in 21 patients accounting for 72.41%. The predominant pattern was granular glomerular basement membrane which was noted in 9 patients (31.03%). The diagnostic utility of IF was noted in 2 cases (6.90%) whose diagnoses included IgA nephropathy and C1q nephropathy. The IF studies helped in modification of the final diagnosis in 1 case (1.72%) whose final diagnosis was lupus nephritis class I.

Conclusion: Immunofluorescence studies have complemented the clinical, histomorphological findings in patients both in primary and secondary glomerular diseases. However, it was even more of diagnostic importance in 5 patients including IgA nephropathy, C1q nephropathy and Lupus nephritis class I where a confident diagnosis could be rendered only because of availability of immunofluorescence studies. Hence, immunofluorescence studies when combined with histomorphologic findings by light microscopy, clinical, biochemical and serological markers can yield a better and precise diagnosis.

Keywords: *Renal Biopsies, Histomorphology, Immunofluorescence*

Introduction

The evaluation of the understanding of medical diseases of the kidney which dwells predominantly upon glomerulopathies, is one of the most fascinating stories in the history of Medicine. There is a rising incidence of kidney disease and it is responsible for high rate of morbidity.[1]

Inflammation of the glomerulus is called glomerulonephritis, while glomerulopathy is a term used for disorders affecting the glomeruli. Glomerulonephritis constitutes nearly 60% of all non-surgical renal diseases and accounts for a substantial number of cases of end stage renal disease[1]. The immunological basis of glomerular diseases involves the deposition of immune complexes in subepithelial, subendothelial or in the mesangium.

Renal biopsy plays a vital part in establishing the diagnosis, prognosis, and response to treatment. Renal biopsies are done to ascertain the diagnosis, rule out other diagnostic possibilities, assessing the activity and chronicity (scarring) of the lesion. Light microscopy is the standard procedure to evaluate kidney biopsies and haematoxylin and eosin stain. Direct immunofluorescence (DIF) on frozen tissue biopsy is the most widely applied method for the detection of immune deposits in the kidney.[2]

The introduction of a safe and reliable percutaneous biopsy method by Iver and Brun in 1951 opened the door to the modern classification of glomerular diseases. The final diagnosis of renal disease is made possible with the interpretation of renal biopsy using light microscopy, immunofluorescence studies and electron microscopy.[2,3]

Light microscopic morphology is assessed by staining the sections with standard stains like haematoxylin and eosin and other stains. When light microscopic appearances are equivocal, immunofluorescence studies may reveal a pattern which enables the glomerular lesions to be identified.[2] Electron microscopy is expensive and may not be feasible in all situations.

The Direct Immunofluorescence is a powerful adjuvant modality for pathological evaluation of renal biopsies. The utility of this technique is limited by cost, site and the time of biopsy and processing factors, history of treatment and nature of the diseases. Correct fixation and processing is critical and the laboratory must be skilled with renal biopsy light microscopy, immunohistochemistry and immunofluorescence methods.

The present study aims to analyse the clinical features in renal diseases and histomorphology of renal biopsy. Also, specific immunofluorescence pattern is studied by applying the panel of immunofluorescent markers IgG, IgA, IgM, C3, C1q and fibrinogen.

Based on the above findings, the etiopathogenesis of renal diseases in the patient is analysed so that better therapeutic strategies can be formulated and administered to improve the clinical outcome of the patient.

Materials and Methods

The present study was conducted in the Department of Pathology, Coimbatore Medical College, Coimbatore from January 2017 to June 2017. A total of 30 cases, two renal core biopsies for each case, one in formalin and other in Phosphate buffer solution were received. The study was performed based on the following proforma.

Inclusion Criteria

Renal biopsy specimens of the patients of all age groups and both sexes with altered renal function suggestive of kidney disease from the Department of Nephrology, Coimbatore Medical College and Hospital, Coimbatore were included in this study.

Exclusion Criteria

1. Specimens not received in phosphate buffer solution for immunofluorescence studies.
2. Specimens that are very tiny for processing and considered inadequate with no glomeruli in subsequent serial sections for light microscopy.
3. Specimens without required clinical and histopathological details.
4. Clinically suspected cases of diabetic nephropathy.
5. Patients that are considered unfit for biopsy (coagulation abnormalities, poor cardiac function).

Indications for Biopsy:

1. All nephrotic syndrome and nephritic syndrome patients who are willing for renal biopsy.
2. Patients with acute renal failure not recovering within 4 weeks of duration.
3. All patients with systemic lupus erythematosus who are willing for biopsy.

Before the procedure a pre-renal anaesthetic assessment including prothrombin time, bleeding time, complete blood count was checked and xylocaine needle test dose was given.

After obtaining informed consent, under local anaesthesia and aseptic precautions, two cores of percutaneous ultrasound guided biopsy specimens of kidney were taken from the patients with altered renal functions. One core was sent in 10% neutral buffered formalin for routine light microscopic examination and other was sent in phosphate buffer solution (pH 7.4) for immunofluorescence studies. The procedure was performed with an informed consent by the clinician as a routine procedure for diagnosis and treatment.

The renal tissue obtained in 10% neutral buffered formalin was kept for fixation for 12 hours to 24 hours and it is then processed and embedded in paraffin. The sections of 3µ to 4µ thickness were cut and stained using haematoxylin and eosin.

The sections for immunofluorescence were cut using cryostat and the procedure done.

Results

The patients were divided into six groups depending on their age at presentation. (Figure 1)

Group 1 : 1-10 years

Group 2 : 11-20 years

Group 3 : 21-30 years

Group 4 : 31-40 years

Group 5 : 41-50 years

Group 6 : 51-60 years

The highest number of patients were in the age group 31years to 40 years (8) which constituted 27.5% of patients followed by the age group 41 years to 50 years (7) which constituted 25.8% of the patients. The mean age was 33.03 years and median were 35 years. The youngest patient was 9 years and the oldest patient was 57 years.

There was equidistributional of patients among males and females. Of the 30 cases, 14 patients were males constituting

48.27% and 16 patients were females constituting 51.27%. The male to female ratio was found to be 0.933:1. The most common glomerular lesion noted in males was diffuse proliferative glomerulonephritis (5 out of 14 cases) and in females was Lupus nephritis (4 out of 15 cases).

Primary glomerulonephritis constituted 79.3% of cases in the present study. Secondary glomerulonephritis constituted 17.24% of cases. Tubulointerstitial nephritis accounted for only 3.45%(Figure 2)

Based on microscopic diagnosis, cases were categorised among primary and secondary glomerulonephritis and tubulointerstitial diseases. Table 1 shows distribution of cases based on microscopic diagnosis.

Of the 30 patients, diffuse proliferative glomerulonephritis constituted highest number of cases accounting for 28.26% (6 cases) followed by membranoproliferative glomerulonephritis accounting for 17.39% (5 cases) overall and also among primary glomerulonephritis. Out of 6 secondary glomerulonephritis cases, Lupus nephritis was the most common lesion noted constituting 70% (4 cases) and all of them were females.

Immunofluorescence studies showed positivity in 21 patients accounting for 72.41%. The predominant pattern was granular staining in glomerular basement membrane which was noted in 9 patients (31.03%).(Table 2).

Granular GBM positivity was noted in 9 patients whose diagnoses included diffuse proliferative glomerulonephritis (2 patients), membranous nephropathy (3 patients), membranoproliferative glomerulonephritis (2 patients) and Lupus nephritis (2 patients).

Non-specific staining in IF was noted in 5 patients whose diagnoses included focal segmental glomerulosclerosis (3 out of 4 cases), Acute tubular necrosis (1 out of 2 patients), sclerosing glomerulonephritis (1 patient), hypertensive glomerulopathy (1 out of 2 patients) and myeloma cast nephropathy (1 patient).

Negative staining was noted in 6 patients whose diagnoses included minimal change disease, diffuse proliferative glomerulonephritis, focal segmental glomerulosclerosis, hypertensive nephropathy, acute tubular necrosis and chronic glomerulonephritis.

No core was obtained for IF in two cases whose diagnoses included mesangioproliferative glomerulonephritis and diffuse proliferative glomerulonephritis.

Out of 30 patients subjected for light microscopy and immunofluorescence studies, the immunofluorescence findings were of diagnostic utility in 2 patients. The final diagnosis was modified based on immunofluorescence findings in 1 patient.

In two patients of mesangioproliferative glomerulonephritis, one of them showed intense mesangial staining of IgA and weak mesangial staining for C3 and the other patient showed intense mesangial staining of C1q. In one patient with light microscopic diagnosis of focal proliferative glomerulonephritis the IF finding was intense mesangial staining of IgA. In another patient with light microscopic diagnosis of focal segmental glomerulosclerosis, the IF findings showed intense mesangial staining of C1q. In these patients the diagnosis was given as IgA nephropathy and C1q nephropathy accordingly. Hence, the diagnostic utility of IF was noted in 4 cases (6.90%).(Figure 3A and Figure 3B)

In a case of minimal change disease, the diagnosis was modified to Lupus nephritis – class I after performing the immunofluorescence studies which showed C3 mesangial staining. Hence the IF studies helped in modification of the final diagnosis in 1 case (1.72%)(Figure4A and Figure 4B)

Table 1: Distribution of cases based on diagnosis.

Final diagnosis	Frequency	Percent
A.Primary glomerulonephritis	(N=23)	79.31
Diffuse proliferative glomerulonephritis	6	28.26
Membranoproliferative glomerulonephritis	5	17.39
Focal segmental glomerulosclerosis	3	15.22
Membranous nephropathy	2	10.87
Minimal change disease	2	10.87
Ig A Nephropathy	1	4.35
C1q Nephropathy	1	4.35
Mesangioproliferative glomerulonephritis	1	4.35
Chronic glomerulonephritis	1	2.17
Sclerosing glomerulonephritis	1	2.17
B.Secondary glomerulonephritis	(N=6)	17.24

Final diagnosis	Frequency	Percent
Lupus nephritis	4	70
Hypertension glomerulopathy	1	20
Myeloma cast nephropathy	1	10
C.Tubular interstitial disease	(N=1)	3.45
Acute tubular necrosis	1	100.0
Total	**(N=30)**	**100.0**

Table 2: Immunofluorescence findings.

Immunofluorescence	Frequency	Percent
Uniform granular staining of GBM	9	31.03
Uniform granular staining of glomerular capillary and mesangial staining	4	12.07
Mesangial staining only	4	12.07
Non-specific staining	5	17.24
Negative	6	22.41
Linear staining of glomerular basement membrane	0	0
No core	2	5.17
Total	**30**	**100.0**

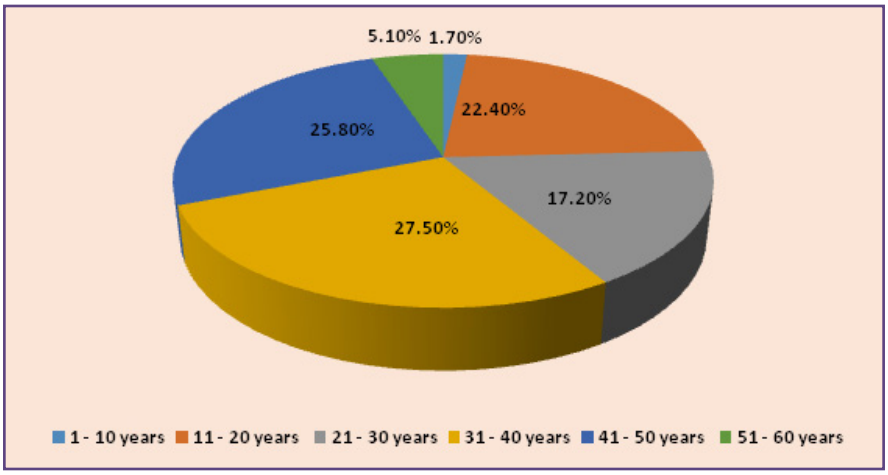

Fig. 1: Age distribution of cases.

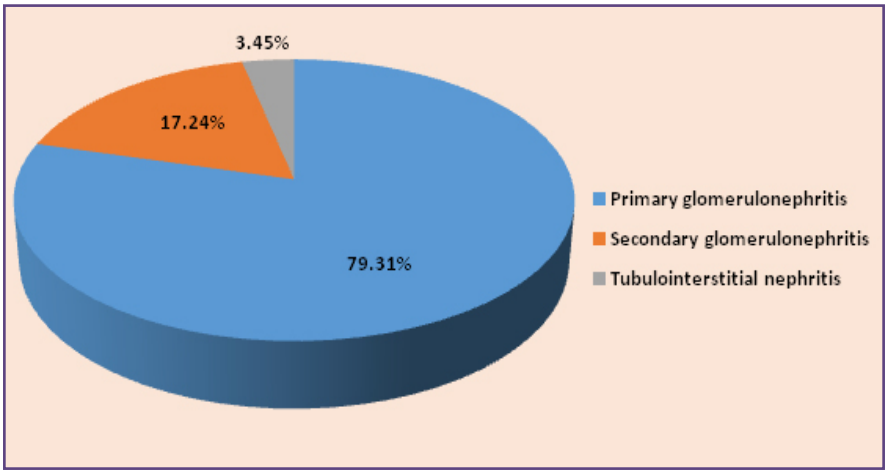

Fig. 2: Distribution of renal diseases.

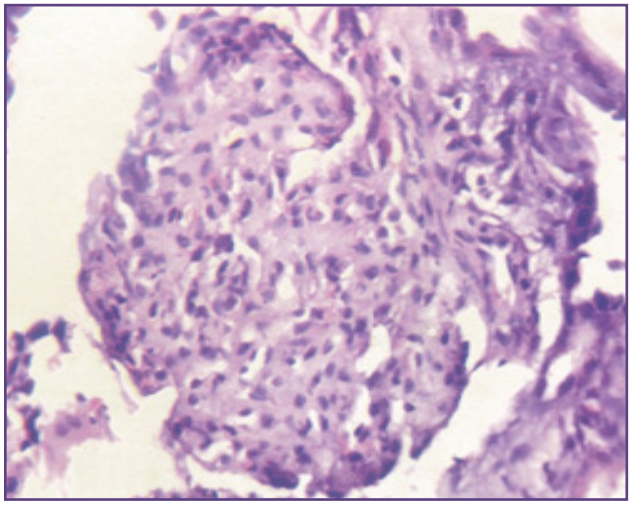

Fig. 3A: IgA nephropathy showing mesangial proliferation. H&E(40x).

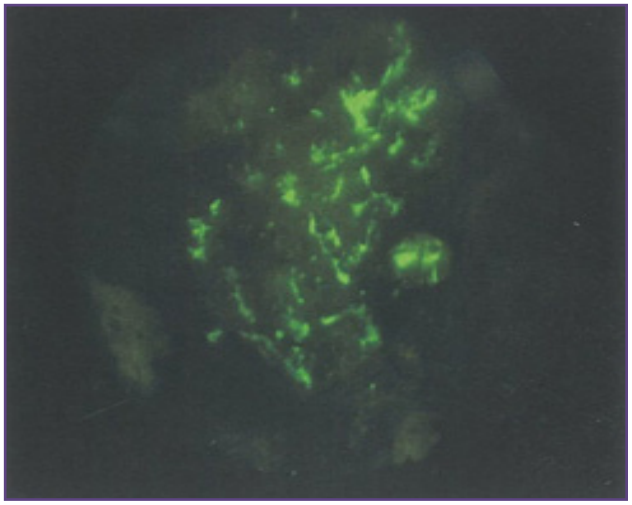

Fig. 3B: Immnunofluorescene of IgA nephropathy showing Ig A deposits in the mesangium. H&E(40x).

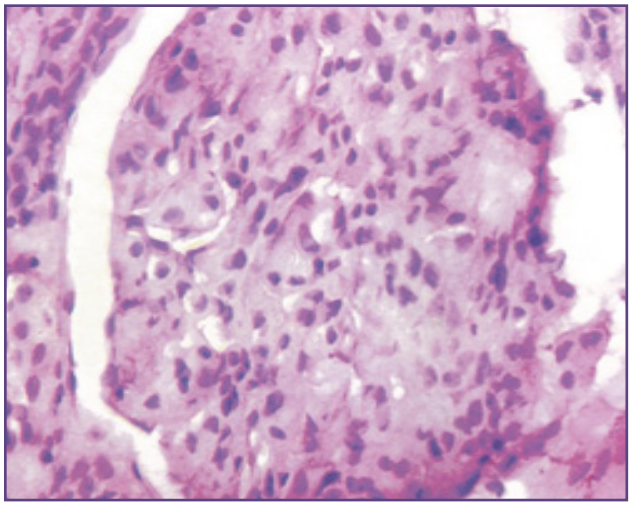

Fig. 4A: Lupus nephritis showing glomerulus with diffuse endocapillary proliferation. H&E.(40x).

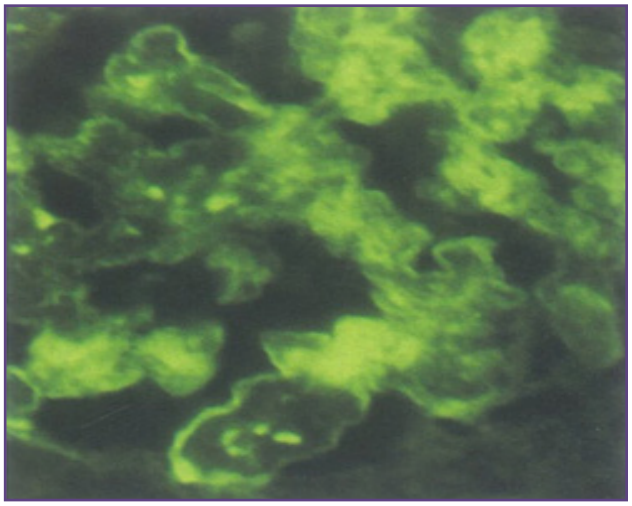

Fig. 4B: Immunofluorescence of Lupus nephritis showing full house pattern-IgG deposits along the basement membrane. H&E(40x).

Discussion

Glomerulonephritis constitutes a major health issue on the health services in developing countries like India. Long term complications are common in these diseases. Early diagnosis and treatment can prevent the morbidity in patients.

The age of patients in this study range from 9 years to 57 years, which is in league with the study by Pierre Simon et al, whose study showed the age range of 10 years to 80 years[4].

In this study, slight female predominance was noted with male to female ratio being 0.933:1. This is in concordance with the study done by Ikechi Okpechi et al, who found the ratio to be 0.824:1[5].

In the present study, primary glomerular lesions constituted 79.31% of the renal diseases which was in concordance with the study by Nasar Yousuf Alwahaibi et al[6]. Diffuse proliferative glomerulonephritis was the most common primary glomerular lesion noted which is in league with study done by Lt Col GU Deshpande et al[7].

Renal biopsy has contributed greatly to a rational classification of intrinsic renal diseases. Light microscopy, Immunofluorescence and Electron Microscopy facilities are needed for correct diagnosis of any glomerular disease. In our study, Immunofluorescence combined with light microscopy helped to give accurate results in most cases despite the lack of availability of Electron Microscopy.

In the present study, secondary glomerular lesions constituted 17.24% of the renal diseases which correlated with Howard et al[8] and Ivan Rychlı´k et al[9] and the most common secondary glomerular lesion was Lupus nephritis which is in concordance with the studies done by Ahmed Al Arrayed et al, Niang Abdu et al, Nasar Yousuf Alwahaibi et al[6] and Ivan Rychlı´k et al[9]. All the patients of lupus nephritis were females.

In the present study, tubulointerstitial nephritis cases constituted 3.45% which is in concordance with the study done by Patricia Malafronte et al[10].

Immunofluorescence has certain advantages. Diseases like IgA nephropathy, IgM nephropathy, C1q nephropathy and anti-GBM glomerulonephritis can only be diagnosed by immunohistology, whereas the diagnosis of other diseases is confirmed and redefined by Direct Immunofluorescence. It is of great use in categorizing crescentic glomerulonephritis into immune complex glomerulonephritis and pauci-immune glomerulonephritis thus facilitating recognition of ANCA associated nephropathy.

Conclusion

The epidemiology of renal diseases differ from developed countries to developing countries. Developing country like ours has shown that the incidence of post infectious glomerulonephritis is still high compared to other glomerular lesions like membranous nephropathy and focal segmental glomerulosclerosis which is more common in developed countries. This can be attributed to the low socioeconomic status, prevalence of infections, lack of awareness regarding health care.

Immunofluorescence studies have complemented the clinical, histomorphological findings in 30 patients including primary, secondary glomerular and tubulointerstitial diseases. However, it was even more of diagnostic importance in 5 patients including IgA nephropathy, C1q nephropathy and Lupus nephritis class I where a confident diagnosis could be rendered only because of availability of immunofluorescence studies. Hence, immunofluorescence studies when combined with histomorphologic findings by light microscopy, clinical, biochemical and serological markers can yield a better and precise diagnosis which can help in improved management of nephrology patients.

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