

Diagnostic Utility of Direct Immunofluorescence in Renal Biopsies with Diabetic Nephropathy

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ABSTRACT

Diabetic nephropathy (DN) is one of the leading causes of chronic kidney disease and end-stage renal disease worldwide, and renal biopsies performed in diabetic patients frequently reveal a broad and overlapping spectrum of glomerular pathology. Light microscopy remains the primary tool for renal biopsy interpretation, but direct immunofluorescence (DIF) provides an essential ancillary means of identifying immune deposits that are not always evident on routine histopathology, helping to differentiate DN from immune-mediated glomerular disease and to detect superimposed renal pathology. This observational study was conducted over 18 months on 42 adequately sampled native renal biopsies, each evaluated by light microscopy and DIF for IgG, IgA, IgM, C3, C1q, kappa and lambda light chains, and fibrinogen. The study population showed a male predominance (73.8%; male-to-female ratio 2.8:1) with a mean age of 42.1 years and peak incidence in the 51-60-year age group. IgA (95.2%), kappa (90.5%), and lambda (88.1%) were the most frequently positive markers overall, and C1q positivity (66.7%) exceeded C3 positivity (42.9%), suggesting frequent classical complement pathway activation. DN was the single most common diagnosis (33.3%), followed by lupus nephritis (11.9%), with membranous nephropathy, membranoproliferative glomerulonephritis, and nephrosclerosis accounting for 7.1% each. Biopsies with DN typically showed negative or weak, nonspecific staining, in clear contrast to the strong, granular “full-house” positivity of lupus nephritis and the granular capillary-wall IgG deposition of membranous nephropathy. These findings confirm that DIF has diagnostic, classificatory, and exclusionary value in the renal biopsy work-up of diabetic patients, and is particularly important for identifying superimposed immune-mediated glomerular disease that would otherwise be missed on light microscopy alone.

Keywords: *diabetic nephropathy; direct immunofluorescence; renal biopsy; immunoglobulin deposition; glomerulonephritis*

INTRODUCTION

Diabetic nephropathy (DN) is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide [1]. As the global burden of diabetes mellitus continues to rise, an increasing number of diabetic patients undergo renal biopsy to characterise the nature of their kidney disease, since proteinuria and renal dysfunction in a diabetic patient may arise from DN alone, from an unrelated non-diabetic renal disease, or from both processes occurring together [2]. Renal biopsy interpretation relies primarily on light microscopy (LM), which allows assessment of glomerular, tubulointerstitial, and vascular architecture and forms

the basis of the widely used Renal Pathology Society classification of DN [3]. LM alone, however, cannot reliably identify immune-complex- or complement-mediated disease processes that may coexist with, or mimic, diabetic glomerulosclerosis. Direct immunofluorescence (DIF) is therefore an important ancillary diagnostic modality that identifies the deposition of immunoglobulins and complement components within the glomerulus, information that is not always evident on routine histopathology. DIF helps differentiate DN from immune complex-mediated disease, complement-mediated disease, and other metabolic or vascular renal lesions, and is of particular value in excluding a superimposed immune-

mediated glomerulonephritis in a diabetic patient with atypical clinical or histological features. Renal diseases often demonstrate overlapping light-microscopic appearances, and in this context DIF is not merely a supportive investigation but one of genuine diagnostic significance. This study was undertaken to evaluate the diagnostic utility of DIF in a series of renal biopsies, with the specific aims of analysing immunofluorescence deposition patterns, correlating DIF findings with the final clinicopathological diagnosis, and assessing the role of DIF in differentiating DN from immune-mediated glomerular lesions.

MATERIALS AND METHODS

Study Design and Setting

This was an observational study conducted in the Department of Pathology, MGM Medical College and Hospital, Kamothe, over a period of 18 months. All native renal biopsies received during the study period were considered for inclusion.

Case Selection

A total of 42 renal biopsies were studied. Biopsies that were inadequate or non-representative of renal parenchyma, for example those containing an insufficient number of glomeruli or predominantly medullary tissue, were excluded from analysis.

Histopathological and Immunofluorescence Evaluation

Every biopsy was evaluated by both light microscopy and direct immunofluorescence. For light microscopy, tissue was processed routinely and stained with haematoxylin and eosin, periodic acid-Schiff, and silver methenamine stains as required. For DIF, a separate core of renal tissue was snap-frozen and

processed using fluorescein isothiocyanate (FITC)-conjugated antisera against IgG, IgA, IgM, C3, C1q, kappa and lambda light chains, and fibrinogen. Sections were examined under a fluorescence microscope, and the intensity, pattern (linear, granular, or mesangial), and glomerular distribution of staining for each marker were recorded. Findings on DIF were correlated with the light-microscopic appearances and the final clinicopathological diagnosis in every case.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize demographic characteristics, frequencies of immunofluorescence marker positivity, and distribution of histopathological diagnoses. Continuous variables were expressed as mean $\pm$ standard deviation, whereas categorical variables were presented as frequencies and percentages. Results were summarized descriptively because the primary objective of the study was to evaluate the diagnostic utility of direct immunofluorescence rather than to compare intervention groups. A two-sided p-value of <0.05 was considered statistically significant wherever applicable.

Sample Size Justification

As this was an observational descriptive study conducted over a predefined study period of 18 months, all eligible native renal biopsy specimens fulfilling the inclusion criteria were consecutively enrolled. Therefore, no formal sample size calculation was performed, and the final sample comprised all available adequately sampled renal biopsies received during the study period.

RESULTS AND DISCUSSION

Demographic Profile

Of the 42 renal biopsies studied, 31 (73.8%) were from male patients and 11 (26.2%) were from female patients, giving a male-to-female ratio of 2.8:1 (Figure 1). The mean age of the study population was 42.13 years, and the highest incidence of biopsy was seen in patients aged 51-60 years, with middle-aged and elderly patients together forming the bulk of the study population. This age and sex distribution reflects the increasing burden of chronic metabolic and immune-mediated renal disease in this demographic group.

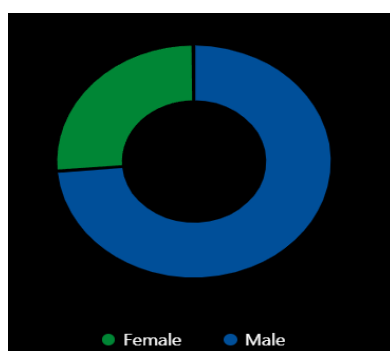


Figure 1: Sex distribution of the study population (n = 42). Male patients accounted for 73.8% of biopsies and female patients for 26.2%, giving a male-to-female ratio of 2.8:1.

Overall Immunofluorescence Findings

On DIF, IgA was the most frequently positive marker, seen in 95.2% of biopsies, followed by kappa (90.5%) and lambda (88.1%) light chains. C1q was positive in 66.7% of cases, IgG in 64.3%, IgM in 57.1%, and C3 in the fewest cases, 42.9% (Table 1, Figure 2). The high and broadly similar frequency of kappa and lambda positivity suggests that the immune deposits identified in this cohort were predominantly polyclonal rather than a manifestation of a monoclonal light-chain disease. Notably, C1q positivity exceeded that of C3, indicating that classical complement pathway activation was identified more often than activation restricted to the alternative or terminal pathway.

Table 1: Frequency of positivity for individual immunofluorescence markers among 42 renal biopsies.

Marker	Positive cases, n (%)
IgA	40 (95.2)
Kappa	38 (90.5)
Lambda	37 (88.1)
C1q	28 (66.7)
IgG	27 (64.3)
IgM	24 (57.1)
C3	18 (42.9)

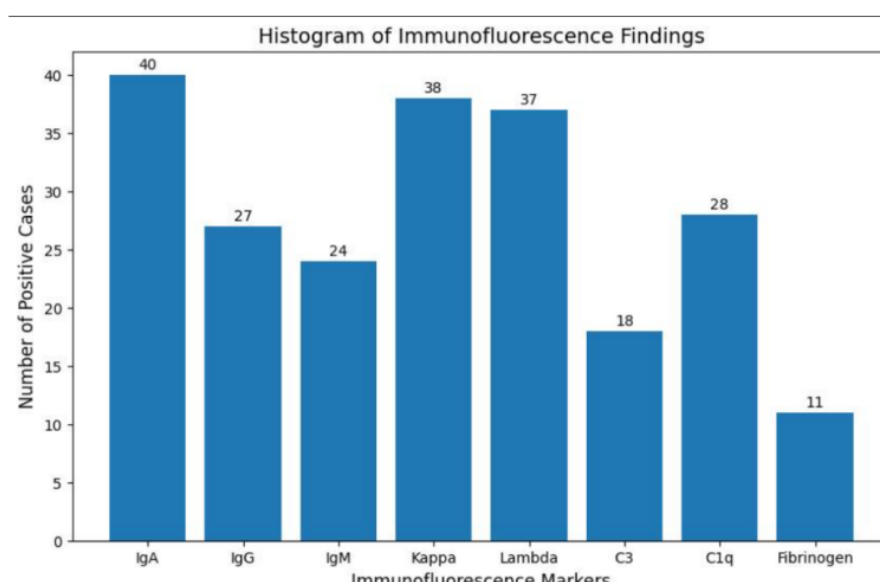


Figure 2: Frequency of positivity for each immunofluorescence marker among 42 renal biopsies. IgA, kappa, and lambda were the most frequently positive markers, while C3 was the least frequently positive.

Disease Prevalence

Diabetic nephropathy was the single most common histopathological diagnosis in this series, accounting for 33.3% of biopsies (14 cases), followed by lupus nephritis (11.9%, 5 cases). Membranous nephropathy, membranoproliferative glomerulonephritis (MPGN), and nephrosclerosis each accounted for 7.1% of cases (3 cases each), and the remaining biopsies were distributed among other renal lesions (Table 2, Figure 3). Although DN formed the largest single diagnostic category, immune-mediated glomerular diseases collectively represented a clinically significant subset of the cohort, underscoring the importance of a systematic DIF work-up in every diabetic patient undergoing renal biopsy [2,6].

Table 2: Distribution of final histopathological diagnoses among 42 renal biopsies.

Diagnosis	n (%)
Diabetic nephropathy	14 (33.3)
Lupus nephritis	5 (11.9)

Membranous nephropathy	3 (7.1)
Membranoproliferative glomerulonephritis (MPGN)	3 (7.1)
Nephrosclerosis	3 (7.1)
Other renal lesions	14 (33.4)

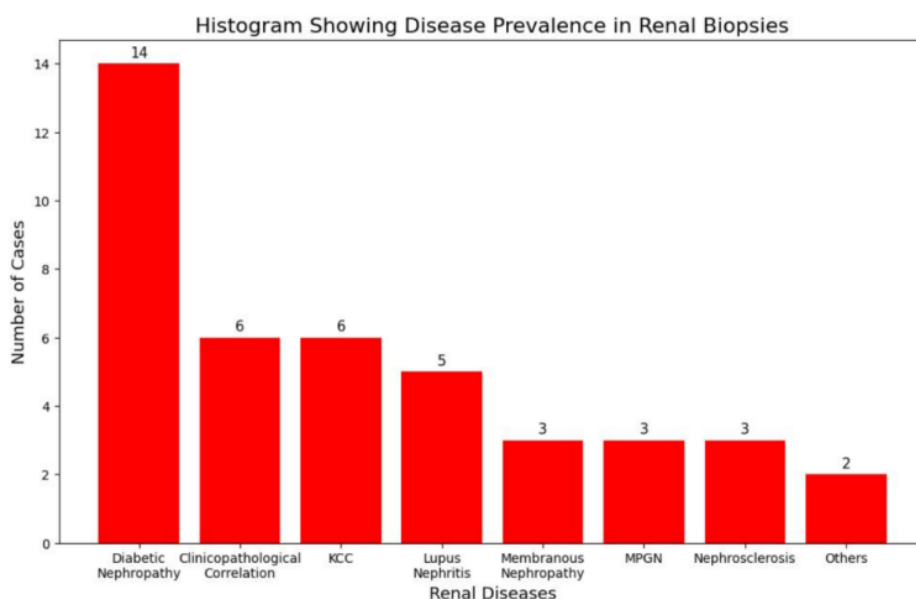


Figure 3: Distribution of final histopathological diagnoses among 42 renal biopsies. Diabetic nephropathy was the single most common diagnosis, followed by lupus nephritis.

Immunofluorescence in Diabetic Nephropathy

In biopsies with a final diagnosis of DN, DIF characteristically showed either negative staining or weak, nonspecific positivity. Occasional cases demonstrated a faint linear pattern of positivity along the glomerular and tubular basement membranes (Figure 4a), consistent with nonspecific trapping of plasma proteins rather than true immune-complex deposition. Mild IgM and C3 positivity was occasionally noted, typically limited to areas of segmental or global glomerulosclerosis (Figure 4b), reflecting passive entrapment within sclerosed capillary loops rather than an immune-mediated process. Significant or strong granular complement deposition was generally absent. These findings support the largely metabolic and non-immune pathogenesis of DN, in which basement-membrane thickening, mesangial expansion, and eventual nodular and global glomerulosclerosis occur independent of immune-complex deposition [3,4]. In this context, DIF becomes valuable chiefly for its exclusionary role, allowing the pathologist to rule out a superimposed immune-mediated glomerular disease in a patient whose biopsy might otherwise be interpreted as pure diabetic glomerulosclerosis on light microscopy alone.

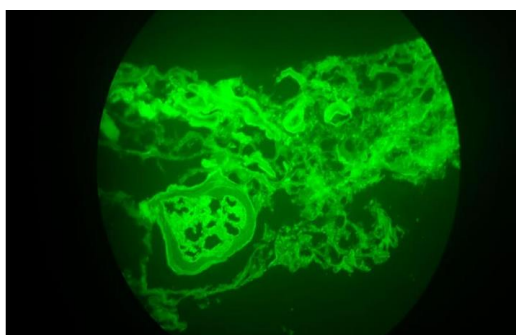


Figure 4a: Direct immunofluorescence photomicrograph from a biopsy of diabetic nephropathy, showing faint linear accentuation of staining along the glomerular and tubular basement membranes, a nonspecific pattern attributable to passive trapping of plasma proteins rather than immune-complex deposition.

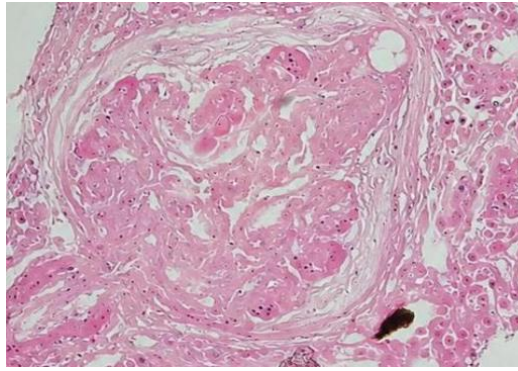


Figure 4b: Light photomicrograph showing global sclerosis with complete obliteration of glomerular capillary loops and marked hyalinization, a typical advanced glomerular lesion of diabetic nephropathy (haematoxylin and eosin).

Comparison with Immune-Mediated Lesions

In marked contrast, biopsies diagnosed as lupus nephritis showed the classic “full-house” pattern of positivity, with strong granular deposition of IgG, IgA, IgM, C3, and C1q along the glomerular capillary walls and mesangium [5]. Membranous nephropathy showed a diffuse granular pattern of IgG along the capillary wall, usually with accompanying, though less intense, C3 positivity, while MPGN biopsies showed a mixed pattern of immunoglobulin and complement deposition, reflecting the immune-complex-mediated pathogenesis of these disorders. Figure 5 illustrates a representative immunofluorescence photomicrograph showing strong, linear IgA positivity, a pattern distinct from the weak and nonspecific staining typical of DN. The strong, disease-specific, and often distinctive deposition patterns seen in these immune-mediated glomerular diseases stand in clear contrast to the weak or absent immunofluorescence typical of DN, and this distinction formed the basis for their differentiation in the present series.

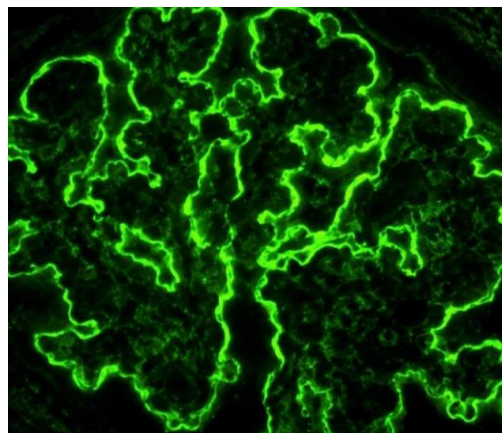


Figure 5: Direct immunofluorescence photomicrograph showing strong linear IgA positivity along the glomerular basement membrane, illustrating a specific pattern of immunoglobulin deposition as seen in an immune-mediated glomerular lesion.

Diagnostic Role of Direct Immunofluorescence

Taken together, the findings of this study illustrate three complementary roles that DIF plays in the evaluation of a renal biopsy from a diabetic patient. First, it is diagnostic, in that it identifies disease-specific patterns of immune deposition that point directly towards a specific glomerular disease. Second, it is classificatory, since the pattern, intensity, and distribution of staining allow glomerular diseases to be sub-classified in a manner that light microscopy alone cannot achieve, as previously demonstrated for lupus nephritis [5] and DN [3]. Third, and perhaps most important in the context of diabetes, DIF is exclusionary, since a negative or nonspecific DIF pattern allows the pathologist to exclude a superimposed immune-mediated glomerulonephritis with reasonable confidence. This exclusionary role is

of particular clinical significance when a diabetic patient presents with atypical clinical or histological features, such as an unusually rapid decline in renal function, heavy proteinuria disproportionate to the duration of diabetes, or an absence of diabetic retinopathy, all of which should prompt a careful search for a non-diabetic renal disease [2,6].

Strengths of the Study

The present study provides a comprehensive clinicopathological evaluation of renal biopsies by integrating light microscopy with direct immunofluorescence, thereby enhancing diagnostic accuracy in diabetic nephropathy. A standardized panel of immunofluorescence markers, including IgG, IgA, IgM, C3, C1q, kappa, lambda, and fibrinogen,

was applied uniformly to all biopsy specimens. The study also highlights the practical diagnostic value of direct immunofluorescence in distinguishing diabetic nephropathy from immune-mediated glomerular diseases and emphasizes its role in identifying superimposed renal pathology that may not be evident on routine histopathology alone.

Limitations of the Study

The present study has certain limitations. First, it was conducted at a single tertiary care center with a relatively small sample size, which may limit the generalizability of the findings. Second, the observational design precluded assessment of long-term clinical outcomes and prognostic significance of the immunofluorescence findings. Third, electron microscopy was not routinely available for all cases, preventing correlation of ultrastructural findings with light microscopy and immunofluorescence. Finally, the study primarily focused on diagnostic utility and did not evaluate treatment response or patient follow-up.

Future Directions

Future multicenter studies involving larger patient populations are warranted to validate these findings across diverse demographic and clinical settings. Incorporation of electron microscopy, immunohistochemistry, and molecular biomarkers may further improve the diagnostic precision of renal biopsy evaluation. Longitudinal studies correlating immunofluorescence patterns with disease progression, therapeutic response, and renal outcomes would provide valuable prognostic information. Additionally, development of standardized diagnostic algorithms integrating clinical, histopathological, immunofluorescence, and molecular parameters may further optimize the management of diabetic patients undergoing renal biopsy.

Clinical Implications

The findings of this study support the routine use of direct immunofluorescence as an essential adjunct to light microscopy in the evaluation of renal biopsies from diabetic patients. Identification of disease-specific immunofluorescence patterns facilitates accurate differentiation between diabetic nephropathy and immune-mediated glomerular diseases, thereby influencing clinical decision-making and therapeutic strategies. Early recognition of superimposed glomerular disorders may enable timely initiation of appropriate immunosuppressive or disease-specific treatment, ultimately improving patient outcomes and reducing the risk of progressive renal dysfunction.

CONCLUSIONS

Direct immunofluorescence plays an important supportive and exclusionary role in the histopathological evaluation of renal biopsies from

diabetic patients. In this series, DN was the single most common diagnosis, and biopsies with DN typically demonstrated absent or nonspecific immune deposits on DIF, in clear contrast to the strong, disease-specific patterns seen in immune-mediated glomerular diseases such as lupus nephritis, membranous nephropathy, and MPGN. DIF is therefore essential for excluding a superimposed immune-mediated glomerular disease in diabetic patients, a distinction that cannot reliably be made on light microscopy alone. Accurate renal diagnosis in this setting requires the integration of clinical findings, light microscopy, and immunofluorescence, and DIF remains indispensable both for disease classification and for a clearer understanding of renal immunopathogenesis in the diabetic patient.

Highlights

- Direct immunofluorescence significantly improves diagnostic evaluation of renal biopsies in diabetic patients.
- Diabetic nephropathy typically demonstrates absent or weak nonspecific immunofluorescence staining.
- Immune-mediated glomerular diseases exhibit characteristic disease-specific immunofluorescence patterns.
- Direct immunofluorescence effectively differentiates diabetic nephropathy from superimposed glomerular diseases.
- Combined assessment using light microscopy and direct immunofluorescence enhances clinicopathological diagnosis and patient management.

Patient Consent for Publication

Not applicable. The Institutional Ethics Committee waived the requirement for individual written informed consent because this study involved retrospective analysis of anonymized archived renal biopsy specimens.

Ethics approval and consent to participate

This study was approved by the Institutional Ethics Committee of MGM Medical College and Hospital, Kamothe (Approval Number: DHR-EC/SC/2024/07/110). As the study involved retrospective evaluation of archived renal biopsy material and did not involve any additional intervention on patients, the requirement for individual written patient consent was waived by the committee. All patient data were anonymised prior to analysis.

List of abbreviations

C1q: complement component 1q; C3: complement component 3; CKD: chronic kidney disease; DIF: direct immunofluorescence; DN: diabetic nephropathy; ESRD: end-stage renal disease; FITC: fluorescein isothiocyanate; GBM: glomerular basement membrane; Ig: immunoglobulin; LM: light microscopy; MPGN: membranoproliferative

glomerulonephritis; TBM: tubular basement membrane.

Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

Souradip Bhaduri conceived and designed the study, performed the immunofluorescence and histopathological evaluation, analysed the data, and drafted the manuscript. Rahul Rajbhar contributed to case collection, light-microscopic evaluation, and data analysis. Shilpi Sahu contributed to immunofluorescence evaluation, data interpretation, and critical revision of the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

Not applicable.

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