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CLINICAL RESEARCH

## Lack of electron microscopy hinders correct renal biopsy diagnosis: A study from India

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### ABSTRACT

Electron microscopy (EM) is performed routinely on all native kidney biopsies in the western world. However, in India, it is not regularly performed due to non-availability and financial constraints. The aim of this prospective study was to evaluate the usefulness of routinely performing EM on native kidney biopsies. In order to eliminate selection bias, all consecutive native kidney biopsies were included in this study, provided they had adequate tissue for light, immunofluorescence (IF), and EM. The biopsies were reported on the basis of light and IF microscopy. EM was performed on each case by another pathologist who also independently reviewed the light microscopic slides and IF images. The findings were then reviewed to assess how the ultrastructural features contributed to the primary diagnosis and assigned to one of the following categories: 1. Crucial for diagnosis, 2. Important contribution, or 3. Not required. Of the 115 cases evaluated, EM was crucial in 12% of the cases. In 20% of the cases, it provided important confirmatory information and in the remaining 68% cases, EM was not considered required. This study supports the use of EM as a routine diagnostic tool in the evaluation of native kidney biopsies. There is an urgent need for availability and accessibility of EM in our country.

### ARTICLE HISTORY

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### KEYWORDS

Electron microscopy;  
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### Introduction

The value of electron microscopy (EM) in the morphological diagnosis of renal diseases is well documented [1,2]. It has contributed immensely to the understanding of the pathogenesis of kidney diseases as it allows the recognition of changes which cannot be observed on light and immunofluorescence (IF) microscopy [3]. EM is routinely being performed on kidney biopsies in many developed countries [4]. On the contrary, due to financial restraints and poor availability, very few centers in developing countries routinely perform EM studies as a part of the evaluation of kidney biopsies [5,6].

The aim of this study was to evaluate the usefulness of routinely performing EM on native kidney biopsies.

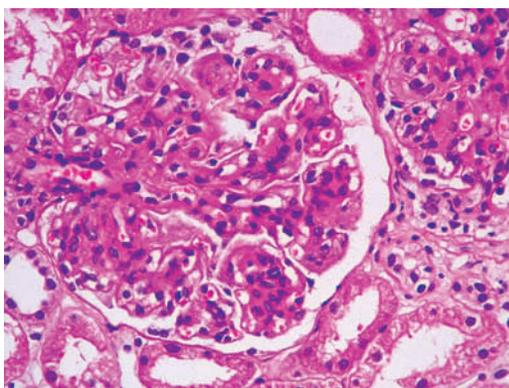
### Materials and methods

This prospective study was done between two institutions, Muljibhai Patel Urological Hospital (MPUH), a tertiary care institute in India, and Nephropath in USA. This study was approved by the ethics committee and review board of MPUH and the institutional review board of Nephropath.

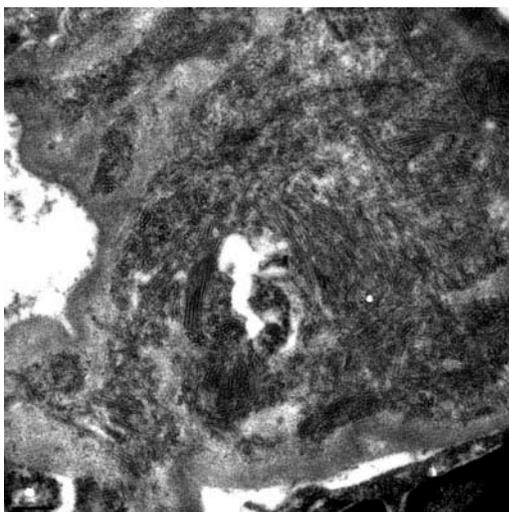
All consecutive native kidney biopsies performed at Muljibhai Patel Urological Hospital (MPUH), a tertiary care institute in India, over a period of 6 months, between November 2008 and April 2009, were included in this study.

Biopsies in which there was insufficient tissue available for light, IF, or EM were excluded from this study. The tissue was determined to be insufficient if there were no glomeruli available for evaluation. A total of 134 native kidney biopsies were performed during the study period, of which 19 had insufficient tissue for evaluation by light, IF, or EM and so were excluded from this study. A total of 115 biopsies were available for this study.

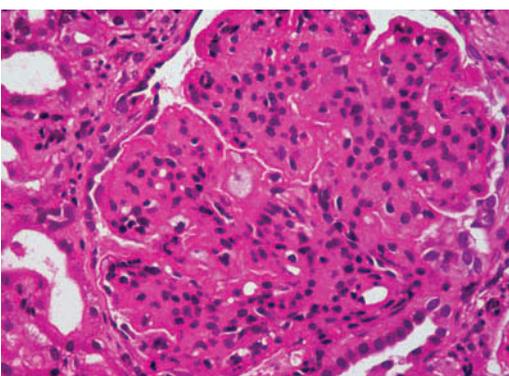
In each biopsy, two cores of renal tissue were obtained using an 18G needle for patients under 12 years and a 16G needle for patients' over 12 years of age. All biopsies were performed under real-time ultrasonography control under local anesthesia. The tissue sampled was immediately assessed using a dissecting microscope, and tissue for EM was cut from one end of a core. This was immediately fixed in 10% neutral buffered formalin, labeled, and stored for shipment. However, if the number of glomeruli was limited, tissue for EM was not cut from the biopsy core. Instead, the tissue in the paraffin block was utilized once the light microscopic study was completed. The tissue for light microscopy was fixed in 10% buffered formalin. The core for IF was placed in phosphate buffer (pH 6.8) and immediately frozen. The tissue for light microscopy was processed as per protocol. Two micron sections, with three to four sections per slide, were taken with two slides each of hematoxylin and eosin, Periodic acid–Schiff, methenamine silver, and one slide of Masson's



**Figure 1.** Immunotactoid glomerulopathy. There is mesangial hypercellularity and matrix expansion, with lobular accentuation by light microscopy.



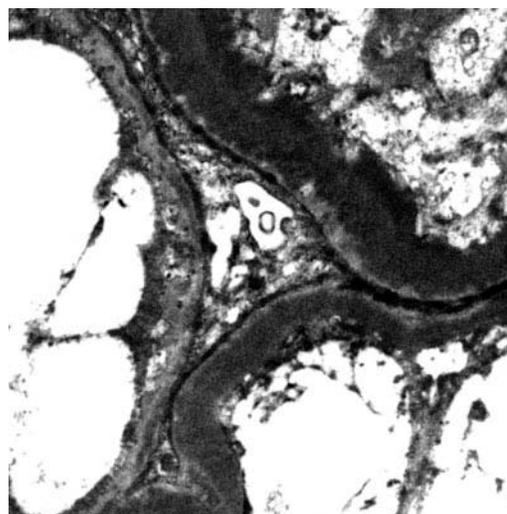
**Figure 2.** Immunotactoid glomerulopathy. Electron micrograph of glomerular microtubular deposits.



**Figure 3.** Dense deposit disease light microscopy shows a membranoproliferative pattern.

trichrome. Direct IF study for C3, IgG, IgM, IgA, C1q, fibrinogen, kappa, and lambda were performed on all cases. A Congo Red stain was performed if indicated.

The pathologist at MPUH reported the biopsy on the basis of light and IF microscopy. The light microscopy slides and



**Figure 4.** Dense deposit disease electron micrograph shows ribbon-like electron-dense deposits in glomerular basement membrane.

the images of IF along with the tissue for EM were sent to Nephropath, in the USA. The clinical details required for interpretation of the biopsy were provided along with each case. An EM was performed on each case by the renal pathologist at Nephropath, who also reviewed the light microscopic slides and IF images. The pathologist in USA was blinded to the report generated by the pathologist in India. The impact of EM in altering the diagnosis was evaluated.

The biopsy was reviewed to assess how the ultrastructural findings contributed to the primary diagnosis and assigned to one of the following categories: 1. Crucial for diagnosis, 2. important contribution, or 3. not required. Electron microscopic study was considered “crucial” for diagnosis when it was needed to make the primary diagnosis. EM was considered as an “important contribution” when it did not alter the primary diagnosis but provided important confirmatory evidence supportive of the primary diagnosis. When the EM did not change the preliminary diagnosis, and was not required to confirm the diagnosis, it was classified as “not required.”

## Results

We evaluated 115 renal biopsies. EM was crucial in 12% of cases. Important confirmatory information was revealed by EM in 20% of cases. Finally, in 68% of cases, EM was not considered “required” (Table 1).

The diagnosis of dense deposit disease in two cases and immunotactoid glomerulopathy in one case required EM for the correct diagnosis. The biggest impact of EM numerically was in our cases of minimal change disease and membranous glomerulopathy (21 of 36 cases either “crucial” or “important”). Infection-associated glomerulonephritis was crucial or important in all four cases.

EM was not considered helpful in lupus nephritis, but lupus is primarily categorized by light and IF findings only [7]. Finally, EM was least important in the tubulointerstitial diseases as might be expected.

**Table 1.** Diagnoses after electron microscopy.

Final diagnosis	n	Crucial	Important	Not required
Minimal change disease	18	2	16	–
Membranous nephropathy	18	3	–	15
FSGS	16	–	–	16
Infection-associated glomerulonephritis	4	2	2	–
Pauci-immune crescentic glomerulonephritis	2	–	–	2
MPGN	2	1	1	–
Cryoglobulinemic glomerulonephritis	1	–	1	–
Dense deposit disease	2	2	–	–
IgA nephropathy	5	–	2	3
Lupus nephritis	6	–	–	6
Hypertensive nephropathy	2	–	–	2
Diabetic nephropathy	6	–	1	5
Amyloidosis	7	–	–	7
LCCN	2	–	–	2
LCCN and MIDD	1	1	–	–
Immunotactoid glomerulopathy	1	1	–	–
Acute tubular injury	12	–	–	12
Acute interstitial nephritis	4	–	–	4
Granulomatous interstitial nephritis	3	–	–	3
Acute pyelonephritis	1	–	–	1
Normal	2	2	–	–
Total	115	14	23	78

Note. FSGS = Focal segmental glomerulosclerosis; MPGN = Membranoproliferative glomerulonephritis; LCCN = Light-chain cast nephropathy; MIDD = Monoclonal immunoglobulin deposition disease.

## Discussion

Several studies have established the importance of EM in the correct interpretation of medical renal biopsies [8–10]. Structural changes in the glomerular basement membrane such as Alport syndrome and thin basement membrane disease can be analyzed only by EM [11]. Early thickening of the basement membrane in diabetic nephropathy can also be seen only on EM [2]. Although IF is sensitive and specific for the presence of immune complexes, EM is required for the exact localization of these deposits and hence offers important information in the diagnosis of immune complex deposition diseases [12,13]. Ultrastructural studies are also required for the diagnosis of diseases with non-immune deposits such as fibrillary and immunotactoid glomerulopathy [14].

Seigel et al., in a series of 213 cases, found that EM was needed for a correct diagnosis in 11% of their renal biopsies and for confirmation or additional information in 36% of their cases [15]. Haas evaluated a series of 233 cases and found that EM was needed to make the final diagnosis in 21%, and it provided important confirmatory data in 21% of the cases [1]. In our study, the results are similar to the previously published data. EM was crucial for diagnosis in 12% of our cases, and in 20% it provided important contribution to the diagnosis.

Pearson et al. [16] found EM useful in differentiating cases of minimal change disease from early membranous nephropathy. A similar observation was made by us in this study where 3 out of the 18 cases of membranous nephropathy would have been wrongly diagnosed as minimal change disease if ultrastructural studies were not performed.

EM was also useful in helping us establish the diagnosis of minimal change disease in 18 (90%) of the 20 cases which had normal-looking glomeruli by light microscopy and a negative IF. The two cases which showed neither foot process

effacement, basement membrane abnormalities nor immune deposits were labeled as normal. A similar observation was made by Wagrowska-Danilewicz [2] where 85.7% of the cases of minimal change disease in his series required EM to establish the final diagnosis.

The essential diagnostic feature for dense deposit disease (DDD) is the demonstration of electron-dense transformation of the glomerular basement membranes by EM [17]. Our series had two cases of DDD and EM was crucial for its diagnosis.

Although ultrastructural studies are not required to make the diagnosis of IgA nephropathy, it can provide important information to support the diagnosis. [18] Demonstration of mesangial electron-dense deposits was helpful in establishing the diagnosis when the IF staining was very weak in two of our five cases of IgA nephropathy.

It may be difficult to distinguish membranoproliferative glomerulonephritis from infection-associated glomerulonephritis. Demonstrating the characteristic subepithelial “humps” in the case of the latter on EM can help in establishing the correct diagnosis [19]. There were four cases of infection-associated glomerulonephritis in our study. An endocapillary proliferative pattern was seen in two of them. The other two cases had a membranoproliferative pattern on light and IF studies. Subepithelial humps were demonstrated in all of them by EM. EM was considered crucial in establishing the diagnosis in the membranoproliferative pattern cases. EM provided important findings which helped us confirm the diagnosis in the other two cases.

Light-chain cast nephropathy (LCCN) is diagnosed on the basis of the morphology of the tubular casts on light microscopy and monoclonality on IF [20]. We had three cases of LCCN, of which three were correctly diagnosed on the basis of light and IF microscopy. The third case proved to be a challenge as we found LCCN coexisting with a monoclonal immunoglobulin deposition disease (MIDD) by EM. EM proved crucial in this case when the subtle powdery deposits were identified on EM in spite of a negative IF study. The glomerular morphologic pattern of MIDD may be that of a minimal change disease, among various other patterns. Also, the abnormal immunoglobulin chains may not be picked up by the commercially available IF antibodies. In these cases, EM provides crucial additional information to establish an unequivocal diagnosis [20].

Light microscopic findings of immunotactoid glomerulopathy are non-specific and fluorescence varies from case to case. The diagnosis of immunotactoid glomerulopathy is not made unless the characteristic hollow microtubules or cylindrical structures are demonstrated ultrastructurally [14]. One patient in our study was found to have immunotactoid glomerulopathy by EM. The biopsy was performed for non-recovery of acute kidney injury (AKI) following post partum hemorrhage. Immunotactoid glomerulopathy was a critical finding even though it was incidental.

The light microscopic appearance of “hyaline thrombi” in the setting of membranoproliferative pattern may be seen in several different glomerular diseases including lupus nephritis, thrombotic microangiopathy, and cryoglobulinemic glomerulonephritis [14]. The EM findings of capillary thrombi and

subendothelial deposits combined with the IF features helped to confirm the case of cryoglobulinemic glomerulonephritis in our study.

By chance this study did not contain any patients with thin glomerular basement membrane syndrome, Alport syndrome, early diabetes, or any of the more rare conditions that are recognized only by EM such as Fabry disease, collagenofibrotic glomerulopathy, and nail-patella syndrome glomerulopathy. Thus, it is likely that our series underestimates, at least somewhat, the percentage of cases requiring EM for diagnosis.

In this series, ultrastructural studies were not of help in the diagnosis of focal and segmental glomerulosclerosis, amyloidosis, pauci-immune crescentic glomerulonephritis, and lupus nephritis. Biopsies with tubulointerstitial or vascular diseases may also have an undiagnosed glomerular lesion like thin basement membrane disease or Alport syndrome which can be diagnosed only by EM. Also, associated foot process effacement can be identified only if EM is performed on these cases. Furthermore, most patients will have single renal biopsy performed in the course of a disease that may be lifelong. It would be desirable to extract as much information as possible from this single investigation.

These features underscore the fact that though EM is considered “crucial” in as many as 20–25% of cases and “important” in another 20–25% of cases, the difficulty is that one cannot predict the cases that would have benefited from the use of EM [1,2,8,9,15]. Thus, the ultimate goal should be the routine use of this technique in all diagnostic kidney biopsies [21,22].

## Conclusion

Our study of a series of renal biopsies for medical renal disease shows that ultrastructural evaluation was either crucial or important to the final diagnosis in 32% of the cases. This data support the use of EM as a routine diagnostic tool in the evaluation of medical renal biopsies.

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