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Current Position of Electron Microscopy in the Diagnosis of Glomerular Diseases

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To establish the role of electron microscopy in the diagnosis of glomerular diseases we reviewed retrospectively 113 renal biopsies. The biopsies were included in this study if tissue was received for light microscopy, immunofluorescence and electron microscopy. The biopsy was assigned to one of the three following categories on the contribution of the ultrastructural findings to the primary diagnosis: essential, important, and not required. Our study revealed that electron microscopy was essential to establish the primary diagnosis in 35 cases (31.0%), was important, but did not alter the preliminary diagnosis in 15 cases (13.3%) and in 63 cases (55.7%) the ultrastructural examination was not needed to confirm the diagnosis. Electron microscopy was essential to create diagnosis in a total of two cases of thin basement membrane disease, in nephropathy in Alport syndrome, in nephropathy in Fabry disease, and was necessary for establishing final diagnosis in 12 cases (85.7%) of minimal lesion. On the basis of electron microscopy it was also possible to establish the precise diagnosis of subtypes in mesangiocapillary glomerulonephritides, describe the stage of membranous glomerulopathy, and find thickening of glomerular basement membrane in the pre-diabetic state. Moreover, ultrastructural examination was helpful to differentiate membranous and mesangiocapillary glomerulonephritis, minimal change nephropathy and early membranous lesions, and distinguish membranous lupus nephritis from idiopathic membranous nephropathy The electron microscopy findings were not of any help in establishing the diagnosis and did not obtain any valuable information in all cases of amyloid nephropathy and IgA nephropathy, as well as in the majority of focal segmental glomerulosclerosis, extracapillary glomerulonephritides, and mesangial proliferative glomerulopathies. In conclusion, the results showed that in 44.3% of glomerulopathies the ultrastructural study provides fundamental or important diagnostic information, and therefore electron microscopy still remains a useful tool in the diagnosis of glomerular diseases.

Introduction

Optimum pathologic evaluation of glomerulonephritis requires light microscopy, immunohistology and electron microscopy. Electron microscopy findings confirm the diagnosis that was rendered by light microscopy and immunohistology, or provide new information that is valuable for patients management. It has often been thought that glomerulopathies can be easily diagnosed by light microscopy and immunofluorescence, but experience has shown that electron microscopy corrects an erroneous conclusion made on the basis of these methods alone. It is generally accepted that the renal biopsy diagnosis require electron microscopy for confirmation if other special investigations are not available or do not work, for example this occurs if the sample for immunofluorescence contains no glomeruli [5]. The main limitations of routine electron microscopy are that it is costly and time consuming [8]. In the past decade, with the increasing pressure to reduce the number of high-cost diagnostic procedures, the number of renal biopsies investigated by electron microscopy in our Department has decreased. In view of the above, the present study was undertaken to evaluate the relevance of ultrastructural studies to the final diagnosis of glomerulopathies.

Materials and Methods

We reviewed retrospectively 113 renal biopsies collected from our pathology database. The biopsies were included in this study if tissue was received for light microscopy, immunofluorescence and electron microscopy

and contained >5 glomeruli for light microscopy evaluation, >2 glomeruli for immunofluorescence and at least one glomerulus for ultrastructural studies. No renal transplant biopsies were included in this study. Laboratory data including urinalysis, 24h protein excretion and serum creatinine level, were collected from each patient. The clinical presentation of the studied glomerulopathies appears from Table 1. For light microscopic examinations the specimens were fixed in 10% buffered formalin, processed and embedded in paraffin. The following stains were used routinely: hematoxylin and eosin (H&E), periodic acid Schiff (PAS) followed by Alcjan Blue, Jones silver methenamine, Masson trichrom's and Congo red. For direct immunofluorescence examination the unfixed specimens were frozen in cryostat at –20°C. Then 5 μm sections were cut on microscope slides and air dried. Direct immunofluorescence was performed using the following immunoglobulin conjugates: anti-IgG/FITC, -IgA/FITC, IgM/FITC, C3/FITC, C1q/FITC, lambda light chains/FITC, and kappa light chains/FITC, all supplied by DakoCytomation. The specimens were examined with the fluorescence microscope (Olympus BX-40). For electron microscopy the specimens were fixed in 3% buffered glutaraldehyde, poststained in 1% osmium tetroxide at 0.1 veronal acetate buffer, processed for electron microscopy and embedded in Epon. Thin sections were cut on Reichert-Jung ultramicrotome. Sections were stained on girds with lead citrate and uranyl acetate and examined with a JEM 100 B electron microscope.

Based on clinical data provided, light microscopy and immunofluorescence findings, a preliminary diagnosis was recorded. After that ultrastructural studies were performed and a final diagnosis was established. The biopsy was assigned to one of the three categories on the basis of how the ultrastructural findings contributed to the primary diagnosis. Essential category group consists of biopsies in which the diagnosis could not have been made without electron microscopy. If the ultrastructural findings did not alter the preliminary diagnosis, however, did provide important information confirming or strengthening the primary diagnosis the biopsies were included into important category group. In not required category group, the electron microscopy was not needed to confirm the diagnosis and did not supply other clinically pertinent information related to diagnosis.

TABLE 1
The clinical presentation of studied glomerulopathies

Clinical presentation	No. of cases
Nephrotic syndrome	32
Nephritic syndrome	11
Acute renal insufficiency	5
Hematuria	19
Proteinuria & hematuria	27
Chronic renal insufficiency & proteinuria & hematuria	12
Chronic renal insufficiency & nephrotic syndrome	7

Results

As appeared from Table 2, electron microscopy was essential for making the diagnosis in 35 cases (31%), important, but did not alter the preliminary diagnosis in 15 cases (13.3%) and in 63 cases (55.7%) the ultrastructural examination was not needed to confirm the diagnosis and did not supply other value information. Within the group in which electron microscopy was essential for diagnosis, the highest percentage of cases consisted of thin basement membrane disease, nephropathy in Alport syndrome (Fig. 1), nephropathy in Fabry disease, mesangiocapillary glomerulonephritis type 1 and 3, as well as

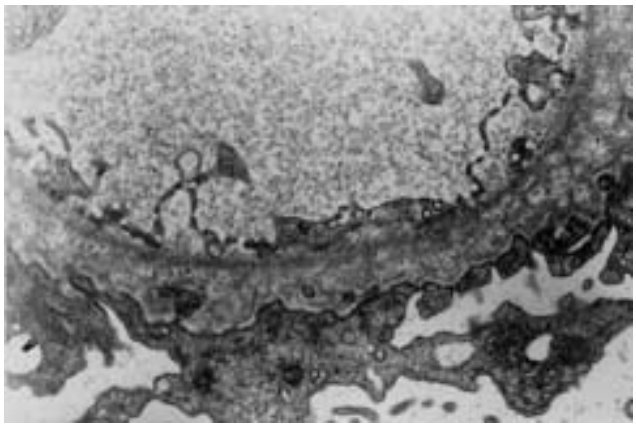

Fig. 1. Lamellation of lamina densa, and thickening of glomerular basement membrane in nephropathy in Alport disease. Magn. 10 000 ×.

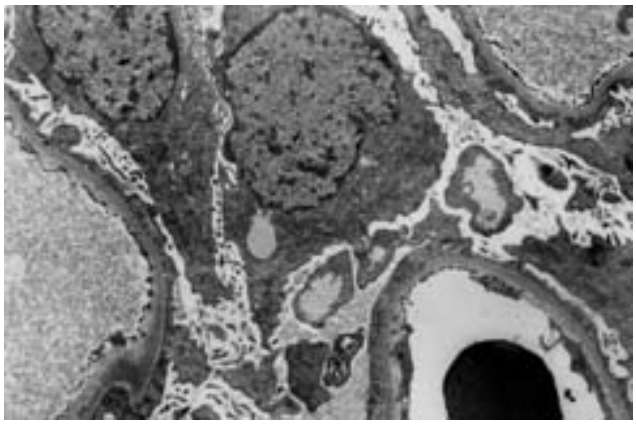

Fig. 2. Diffuse effacement of podocyte foot-processes and microvillous transformation of podocytes in minimal change disease. Magn. 4000 ×.

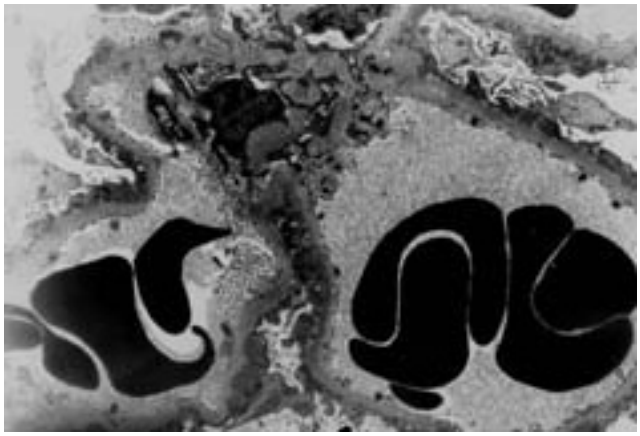

Fig.3. Early stage of membranous glomerulopathy with epimembranous deposits and focally effacement of podocyte foot-processes. Magn 3000 ×.

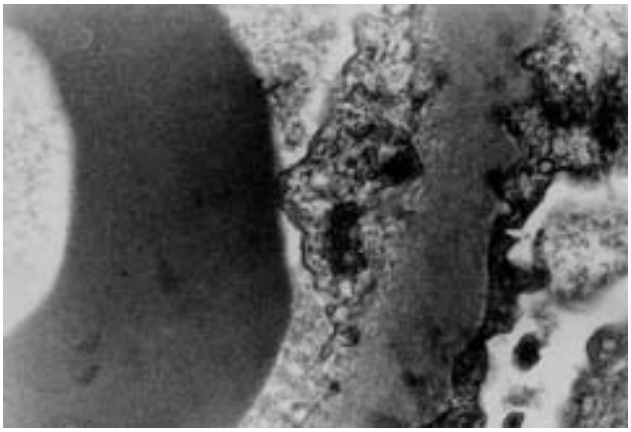

Fig.4. Endothelial tubuloreticular inclusions in V class of lupus nephritis. Magn. 15 000 ×.

TABLE 2
Value of electron microscopy findings in diagnosis of individual types of glomerulopathies

Final diagnosis	No of cases	Essential No. (%) of cases in this category	Important No. (%) of cases in this category	Not required No. (%) of cases in this category
Minimal change disease	14	12 (85.7%)	2 (14.3%)	0
Focal segmental glomerulosclerosis	18	1 (5.6%)	2 (11.1%)	15 (83.3%)
Membranous glomerulopathy	12	3 (25.0%)	3 (25.0%)	6 (50.0%)
IgA Nephropathy	16	0	0	16 (100%)
Mesangial proliferative glomerulonephritis (non IgA)	13	2 (15.4%)	3 (23.1%)	8 (61.5%)
Mesangiocapillary glomerulonephritis- subtype 1	7	7 (100%)	0	0
Mesangiocapillary glomerulonephritis- subtype 3	2	2 (100%)	0	0
Extracapillary glomerulonephritis	4	0	1 (25.0%)	3 (75.0%)
Lupus nephritis	14	3 (21.4%)	3 (21.4%)	8 (57.2%)
Amyloid nephropathy	4	0	0	4 (100%)
Diabetic nephropathy	5	1 (20.0%)	1 (20.0%)	3 (60.0%)
Alport nephropathy	1	1 (100%)	0	0
Thin basement membrane disease	2	2 (100%)	0	0
Fabry disease	1	1 (100%)	0	0
Total	113	35 (31.0%)	15 (13.3%)	63 (55.7%)

minimal change disease (Fig. 2). Ultrastructural examination of renal biopsy was also essential in 25% of membranous glomerulopathies (Fig. 3), 21.4% of lupus nephropathies (Fig. 4), 20% of diabetic nephropathies, 15.4% of mesangial proliferative glomerulopathies and only in 5.6% of focal segmental glomerulosclerosis. In the important category group the ultrastructural finding provides important information confirming or strengthening the primary diagnosis in 25% of membranous glomerulopathies, 25% of extracapillary glomerulonephritides, 23.1% of mesangial proliferative glomerulopathies, 21.4% of lupus nephropathies, 20% of diabetic nephropathies, 14.3% of minimal change nephropathies, and 11.1% of focal segmental glomeulosclerosis. The electron microscopy study was not needed to establish the diagnosis in all cases of amyloid nephropathies and IgA nephropathies, in 83.3% of focal segmental glomerulosclerosis, 75% of crescentic glomerulonephritides, 61.5% of mesangial proliferative glomerulopathies, 60% of diabetic nephropathies, and 57.2% of lupus nephropathies.

Discussion

In our study the highest percentage of cases in essential category group were thin basement membrane disease, nephropathy in Alport syndrome and nephropathy in Fabry disease. Moreover, on the basis of electron microscopy it was possible to establish the precise diagnosis of subtypes in mesangiocapillary glomerulonephritides. Siegel et al [17] pointed out that electron microscopy contributed to diagnosis in 48% cases, but Sementilli et al [15] concluded that on the basis of light microscopy and immunofluorescence findings it is possible to diagnosis about 82–90% of glomerulopathies, however ultrastructural study are essential in diagnosis of hereditary nephropathies. It is generally accepted that in the hereditary nephropathies, electron microscopy makes the definitive diagnosis. Most definitions of thin basement membrane disease in adults require the thinning to be less than 250 nm or less than 200 nm in over 50 percent of glomerular capillaries [3]. In children, thinning below 200 nm is the diagnostic criterion used by Gubler et al. [6]. In Alport nephropathy glomerular basement membrane shows irregular foci of thickening alternating with attenuation, with pronounced splitting and lamination of the lamina densa, often with a basket-wave appearance [12]. Differentiation Alport syndrome from thin-basement membrane disease can be difficult because the two diseases are closely related, however the major abnormalities of the glomerular basement membrane are visualized only by electron microscopy [11]. Similarly, the diagnosis of Fabry disease is done by ultrastructural examination of renal biopsy that documents typical inclusion bodies in the cytoplasm with concentric lamellation and a zebra or onionskin appearance [16]. A typical example of kidney disease in which electron microscopy is essential in diagnostics is minimal change disease Rivera et al. [14] investigated the role of the ultrastructural study in the diagnosis of childhood nephrotic syndrome and found that the contribution of the electron microscopy examination to final diagnosis was essential in 73% of the series. In our study electron microscopy was necessary for making final diagnosis in 12 cases (85.7%) of minimal lesion, whilst the ultrastructural examination was important to confirm the diagnosis in 2 cases of this glomerulopathy. In renal biopsies in adults with minimal change nephropathy light microscopy findings may contain mesangial hypercellularity and even increased mesangial matrix, as well as focal interstitial fibrosis. In these cases the electron microscopy is mandatory to establish the proper diagnosis. It is well known that subtype 1, 2 and subtype 3 of mesangiocapillary glomerulonephritis can be differentiated by ultrastructural examination, only. In our study electron microscopy evaluation of renal biopsies revealed in 7 cases mesangiocapillary glomerulonephritis subtype 1, and in 2 other cases mesangiocapillary glomerulonephritis subtype 3, as defined by Burkholder [1]. We did not observed dense deposit disease. It is worthy of note that a few glomerular diseases have light microscopic and immunofluorescence features that are shared with many types of glomerulonephritis, e.g, fibrillary glomerulonephritis, immunotactoid glomerulopathy and collagenofibrotic glomerulopathy. These diseases can only be conclusively diagnosed by electron microscopy, but we did not find ultrastructural lesions related to the mention above diseases.

Ultrastructural evaluation of renal biopsy specimens was also essential in more then twenty percentages of membranous glomerulopathies and lupus nephropathies, twenty percentages of diabetic nephropathies, and in about fifteen percentages of mesangial proliferative glomerulopathies. In these cases light and immunofluorescence findings were not characteristic and did not resemble lesion typical for these glomerulopathies. It must be stressed that electron microscopy resulted in change in preliminary diagnosis in several our cases. In one case in man with nephrotic syndrome light microscopy showed hypercellular glomeruli with focally thickened glomerular capillary loops and increased mesangial matrix. Immunofluorescence revealed strong granular staining of IgG and C3 in the glomerular capillaries and trace C3 in mesangium. By light microscopy and immunofluorescence the lesion appeared to be mesangiocapillary glomerulonephritis, however ultrastructural studies showed epimembranous and intramembranous deposits, with no subendothelial and mesangial deposits, ruling out the preliminary diagnosis. The second case involved women with the onset of renal failure and nephrotic syndrome. Light microscopy showed focal and segmental glomerular sclerosis and mild hypercellularity. Immunofluorescence showed granular staining of C3 and IgM in sclerotic glomerular area. Primary diagnosis was focal segmental glomerulosclerosis, but ultrastructural study revealed subepithelial deposits along capillary walls resembling membranous glomerulopathy. The third case involved a man presenting with severe proteinuria and hematuria. Light microscopy revealed hypercellular glomeruli with thickened and focally double glomerular basement membrane. Immunofluorescence demonstrated strong staining of C3 and IgG in mesangium and scanty granular staining of C3 along capillary loops. The preliminary diagnosis was mesangiocapillary glomerulonephritis; however no subendothelial deposits were seen by electron microscopy. Ultrastructural study showed deposits localized solely to mesangium ruling out a diagnosis of mesangiocapillary glomerulonephritis. The evaluation of renal biopsy specimens without access to electron microscopy results in missed diagnoses. Skjorten

and Halvarsen [18] found that electron miscroscopy altered the diagnosis in 34% cases. The present data support thesis that electron microscopy made a significant contribution to the precise diagnosis of glomerular disease. In 21 cases (18.6%) the ultrastructural findings did not alter the preliminary diagnosis, however did provide important information confirming or strengthening this primary diagnosis. On the basis of ultrastructural study it was possible to definitely differentiate membranous and mesangiocapillary glomerulonephritis. Electron microscopy evaluation clearly described the stage of membranous glomerulonephritis according to Ehrenreich and Churg [4]. Thickening of basement membrane in diabetes mellitus may be seen in electron microscopy before clinical signs of diabetic renal disease [10]. The ultrastructural findings were also useful in several cases in distinguishing minimal change nephropathy from early membranous lesions that did not show capillary loop thickening by light microscopy and lack of convincing immunofluorescence findings. In membranous glomerulonephritis, the diagnosis may be difficult in stage I of the disease since, at beginning the changes are not evident. The presence of mesangial, subepithelial and intramembranous deposits concomitant with the evidence of tubuloreticular inclusions helped us to distinguish membranous lupus nephritis (V class WHO) from idiopathic membranous nephropathy. In our case the ultrastructural finding was very suggestive for lupus nephritis, and laboratory data obtained 3 weeks later confirmed SLE. Study of Herrera [8] pointed to the value of electron microscopy in diagnosis and clinical management of lupus nephritis

The electron microscopy findings were not of any help in establishing the diagnosis and did not obtain any valuable information in 63 (55.7%) of renal biopsies. It is noteworthy that all cases of amyloid nephropathy and IgA nephropathy were included in this group. The diagnosis of amyloid nephropathies was established by positive Kongo red staining followed by evidence of brifiringerence in the polarization. The diagnosis of IgA nephropathy was definitely established by immunofluorescence on the basis of dominant strong mesangial IgA staining, and/or less C3 staining in the same localization. In contrary to our findings, Gu and Herrera [7] suggest that electron microscopy can be crucial in providing information to support a diagnosis of IgA nephropathy. In 15 cases of focal segmental glomerulosclerosis and 6 membranous glomerulopathy cases light microscopy and immunofluorescence findings were typically for these glomerulopathies, and therefore the ultrastructural examination was not important in making the diagnosis. In 8 cases of lupus nephritis light microscopy and immunofluorescence clearly showed lesions characteristic for WHO class IV-G A/C. In 1 case of extracapillary glomerulonephritis immunofluorescence findings were evident and showed linear staining of IgG along capillary loops, whilst two other ANCA-positive cases were diagnosed as pauci-immune crescentic glomerulopathies. Our results generally are in concordance with the results of others. In study of Haas et al [8] ultrastructural findings were crucial for diagnosis in 21%, important in 21% and not required in 58% cases of glomerulopathies. Pearson et al [13] concluded that only in 25% of cases electron microscopy was unhelpful for diagnosis. Similarly, in study of Collan et al. [2], only about 25% of the electron microscopy reports did not have any influence on the diagnostic process. It must be stressed that, electron microscopy helps localize deposits, detects extremely small deposits, and documents alterations of cellular and basement membrane structure in glomerular diseases. In immune-complex glomerulopathies, the presence of mesangial deposits, in the absence of capillary wall deposits most often corresponds to mild proliferative lesions, whilst a numerous subendothelial deposits usually are found in active, severe glomerular proliferative changes.

In conclusion, the results showed that in about 44% of glomerulopathies the ultrastructural study provides fundamental or important diagnostic information, and therefore electron microscopy still remains a useful tool in the diagnosis of glomerular diseases.

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