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Role of Electron Microscopy in Evaluation of Native Kidney Biopsy

A Retrospective Study of 273 Cases

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Introduction. Electron microscopy (EM) has been widely utilized in the evaluation of kidney biopsies. However, few recent reports have critically assessed its diagnostic value. The aim of this study is to assess the role and value of EM in the evaluation of native kidney biopsies at our institution.

Materials and Methods. A retrospective evaluation of 273 native kidney biopsies performed at our institution over 7 years was done by 2 renal pathologists in order to assess the contribution of EM to the final diagnosis in the knowledge of the light microscopy and immunofluorescence findings.

Results. Electron microscopy had an important diagnostic contribution in 39% of cases, in 17% of which EM was essential for diagnosis. Electron microscopy was essential in the diagnosis of minimal change disease, hereditary nephritis, fibrillary glomerulonephritis, and certain classes of lupus nephritis.

Conclusions. In a great percentage of kidney biopsies, it was possible to make the diagnosis with certainty based on light microscopy and immunofluorescence findings alone. However, still there are numbers of cases in which EM is essentially needed to reach definitive diagnosis. Therefore, at least a piece of tissue should be kept for EM in appropriate fixative in each case, which could then be performed at the discretion of the pathologist.

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INTRODUCTION

Electron microscopy (EM) has been used for the morphological diagnosis of glomerular diseases for more than 3 decades, and its value has been strongly emphasized.[1] In major medical centers where native kidney biopsies are performed, EM is routinely done together with light microscopy and immunofluorescence study for the evaluation of the specimens. Some investigators had observed that about 85% of kidney biopsies had an indication of EM for diagnostic confirmation.[2] Routine EM has proved to be of high value for nephrotic syndrome, classification of glomerular diseases, and also for therapeutic monitoring.[3] In addition, over the past 25 years, several new glomerular diseases have been discovered including, human immunodeficiency syndrome (HIV), fibrillary glomerulonephritis, and C1q nephropathy, in which ultrastructural findings are useful in establishing the diagnosis.[4-6]

However, the use of EM in other areas has markedly declined. In addition, the costs of routine use of EM made the selection of cases that need this diagnostic tool quite rigorous. The aim of this study was to re-evaluate the routine use of EM for native kidney biopsies also examined by light microscopy and immunofluorescence, and to assess whether a more selective approach could be adopted.

MATERIALS AND METHODS

All kidney biopsy specimens taken at King Abdul-Aziz University Hospital between 2000 and 2007 were retrieved from the hospital records. A total of 273 cases were re-examined. All of the cases had immunofluorescence study and EM performed. The light microscopy, immunofluorescence study, and EM findings were reviewed by 2 renal pathologists. Each case was first analyzed using light microscopy and immunofluorescence study, together with the clinical and laboratory data. These findings then were re-evaluated together with the EM findings in order to determine the impact of the EM on the diagnosis of the glomerular disease.

The contribution made by EM was divided into the following categories: A (essential), cases in which electron microscopy was needed to make the primary final diagnosis, either changing the preliminary diagnosis or resolving a differential diagnosis in cases where a firm preliminary diagnosis could not be made; B (helpful), cases in which the ultrastructural findings did not alter the preliminary diagnosis and were not essential in making the primary final diagnosis (however, the EM findings did provide important information related to this primary diagnosis); and C (not required), EM did not change the primary diagnosis and did not supply other clinically important information related to the primary final diagnosis.

RESULTS

The distribution of pathological findings in the 273 cases studied is presented in Table 1. In 167 cases (61.2%), EM was not required to make the diagnosis. Electron microscopy was considered essential in making the diagnosis in 45 cases (16.5%), including 29 cases (10.6%) in which a firm preliminary diagnosis could not be reached with light microscopic and immunofluorescence findings alone. These include cases of minimal change disease, membranoproliferative glomerulonephritis type I and II, fibrillary glomerulonephritis, C1q nephropathy, Alport syndrome, and thin-basement membrane disease.

In case of lupus nephritis, EM was important to establish the diagnosis of class V and combined classes. In addition, EM was helpful in confirming the diagnosis or providing additional clinically relevant information in 61 cases (22.3%). These included cases of focal and segmental glomerulosclerosis, membranous nephropathy, and amyloid nephropathy. Table 2 summarizes the results.

Table 1. Diagnostic Categories of 273 Kidney Biopsies Included in Study

Diagnostic Categories	Number (%)
Class II lupus nephritis	10 (3.7)
Class III lupus nephritis	2 (0.7)
Class IV lupus nephritis	51 (18.6)
Class V lupus nephritis	12 (4.4)
Class VI lupus nephritis	1 (0.4)
Combined class VI + V lupus nephritis	3 (1.1)
Focal and segmental glomerulosclerosis	38 (14.0)
IgM nephropathy	37 (13.6)
Membranous nephropathy	28 (10.3)
Minimal change disease	7 (2.6)
IgA nephropathy and Henoch-Schonlein purpura	20 (7.4)
Post-infectious glomerulonephritis	20 (7.4)
Membranoproliferative types I and II	7 (2.6)
Crescentic glomerulonephritis	6 (2.2)
Fibrillary glomerulonephritis	9 (3.0)
Amyloid nephropathy	2 (0.7)
Diabetic nephropathy	2 (0.7)
Hypertensive nephropathy	2 (0.7)
C1q nephropathy	2 (0.7)
End-stage renal disease	3 (1.1)
Alports syndrome	3 (1.1)
Thin-basement membrane	1 (0.4)
Interstitial nephritis and pyelonephritis	4 (1.5)
Acute tubular necrosis	1 (0.4)
Thrombotic mico-angiopathy	2 (0.7)
Total	273 (100)

DISCUSSION

The role of EM in pathologic examination of specimens from kidney biopsy is well established. Several studies have evaluated routine use of EM in kidney biopsy evaluation, the majority of which were performed during late 1960s and early 1970s.[1-3,7-9] Tighe and Jones described the EM findings in a series of 100 cases and found it to be most useful in distinguishing cases of minimal change nephropathy from early membranous nephropathy and other glomerular disease causing the nephrotic syndrome.[2] They emphasized that the main limitations of routine EM are that it is costly and time consuming. The largest study during that time was done by Siegel and colleagues who evaluated the use of routine EM in 213 kidney biopsy specimens and concluded that EM contributed to diagnosis or patient management in 48% of cases.[7]

Table 2. Contribution of Electron Microscopy to Diagnostic Categories in the Study

Final Diagnosis	Total	Electron Microscopy Contribution Categories*		
		A (%)	B (%)	C (%)
Class II lupus nephritis	10	0	0	10 (100)
Class III lupus nephritis	2	0	0	2 (100)
Class IV lupus nephritis	51	1 (2)	0	50 (98)
Class V lupus nephritis	12	3 (25)	0	9 (75)
Class VI lupus nephritis	1	0	0	1 (100)
Combined class VI + V lupus nephritis	3	3 (100)	0	0
Focal and segmental glomerulosclerosis	38	0	29 (76)	9 (24)
IgM nephropathy	37	0	0	37 (100)
Membranous nephropathy	28	0	28 (100)	0
Minimal change disease	7	7 (100)	0	0
IgA nephropathy and Henoch-Schonlein purpura	20	1 (5)	0	19 (95)
Post-infectious glomerulonephritis	20	7 (35)	0	13 (65)
Membranoproliferative types I and II	7	7 (100)	0	0
Crescentic glomerulonephritis	6	2 (33)	0	4 (67)
Fibrillary glomerulonephritis	9	9 (100)	0	0
Amyloid nephropathy	2	0	2 (100)	0
Diabetic nephropathy	2	1 (50)	0	1 (50)
Hypertensive nephropathy	2	0	0	2 (100)
C1q nephropathy	2	2 (100)	0	0
End-stage renal disease	3	1 (33)	0	2 (67)
Alports syndrome	3	3 (100)	0	0
Thin-basement membrane	1	1 (100)	0	0
Interstitial nephritis and pyelonephritis	4	0	0	4 (100)
Acute tubular necrosis	1	0	0	1 (100)
Thrombotic mico-angiopathy	2	0	2 (100)	0
Total	273	45 (17)	61 (22)	167 (61)

*A indicates electron microscopy is essential for diagnosis; B, helpful; and C, not required.

They found in about one out of 10 cases that EM resulted in a substantially different diagnosis than that suggested by light microscopy alone. They pointed out that it is usually not possible to predict on light microscopy alone those cases where EM would be of most benefit, and so they concluded that this method of examination should be used on a routine basis. Similarly, Olsen and coworkers found that EM results had altered the diagnosis of the light microscopy alone in 11 (13%) of 91 kidney biopsies.[8] However, all of these studies were conducted without the aid of immunfluorescence microscopy which was first utilized by Berger in its original description of IgA nephropathy in 1968,[10] and then became as a routine part of kidney biopsy evaluation 5 to 10 years later.

The 1st study that combined immunfluorescence and EM was in 1977 by Dische and Parsons who described the contribution of immunofluorescence and EM to the diagnosis of glomerulonephritis in 134 cases and concluded that it was essential to complement light microscopy, preferably with immunofluorescence and EM.[11] In 1981, Skjorten and Halvorsen re-evluated the use of semi-thin resin sections and EM in kidney biopsy diagnosis in 200 cases.[12] Electron microscopy altered the diagnosis in 34% of cases and yielded additional useful information in another 45% of cases. They concluded that EM should be used routinely in suspected cases of glomerulonephritis; however, its value in other kidney diseases is less clear, and its use should be decided according to available resources.

Pearson and associates in 1994 studied 88 kidney biopsies.[3] They used EM together with light microscopy and immunofluorescence and found EM to be useful in 5%, essential in 25%, and of no use in 25% of cases. In their study, EM was found to be most useful in both the diagnosis of minimal change nephropathy and its differential diagnosis. In a study by Hass in 1997, of 233 cases of native kidney biopsy,[13] EM was necessary to make the final diagnosis in 21% and provided an important confirmatory data in

another 21% of cases. Diagnoses that required EM included minimal change nephropathy, early diabetic nephropathy, membranous lupus nephritis, membranoproliferative glomerulonephritis, thin-basement membrane nephropathy, and HIV-associated nephropathy. He concluded that if EM cannot be performed routinely on all kidney biopsies, tissue for ultrastructural studies be set aside in each case. In 2004, a study by Sementilli and colleagues[14] of 200 kidney biopsies using light microscopy, immunofluorescence, and EM found that the final diagnosis can be made with the use of light microscopy plus immunofluorescence alone in 77% of cases. Electron microscopy was essential for diagnosis only in 10% and useful in 5.5%.

In the current study, the routine use of EM in conjunction with the light microscopic and immunofluorescence findings was considered to be essential in reaching a definitive diagnosis in 17%, helpful in 61%, and not contributory in 22 %. These results are comparable to what previously cited and very similar to the results obtained by Collan and coworkers,[15] who found EM to be essential for diagnosis in 18.3%, clearly contributed in 53.5%, and of no influence on the final diagnosis in 28.2% of 82 cases of kidney biopsies performed for primary kidney diseases. The result is also very close to a recent study from Egypt by Elhefnawy[16] on 120 kidney biopsy specimens, in which the EM was essential for the diagnosis in 25% of cases, useful in 41.7%, and unhelpful in 33.3% of cases.

Electron microscopy was considered most useful in the current study in the diagnosis of minimal change disease, mainly in its differential diagnosis from other disease that can have normal morphology by light microscopy, such as early membranous nephropathy. In addition, EM was considered essential in establishing the diagnosis of glomerular basement membrane abnormalities including thin-basement membrane disease and Alport syndrome. In these diseases, light microscopy can be normal at first and immunofluorescence is always negative, but EM allows the detection of the alteration at glomerular basement.

For fibrillary glomerulonephritis, EM is required for the diagnosis by demonstrating the randomly arranged straight and nonbranching fibrils in the mesangium or capillary loops or both.[5] These fibrils range from 10 nm to 30 nm in diameter. Similar to fibrillary glomerulonephritis, EM findings are the defining criteria for the diagnosis of immunotactoid glomerulopathy by identifying glomerular deposits of microtubules with focal parallel alignment ranging in diameter from 25 nm to 90 nm.[5]

In cases of primary focal and segmental glomeruoscerosis, the diagnosis can usually be made based on the light microscopy and the immunofluorescence findings of trapping of IgM and complement 3 in the sclerosed glomeruli. Electron microscopy is useful for confirming the diagnosis and also in excluding secondary causes of sclerosis by absence of electron-dense deposits. In the idiopathic focal and segmental glomerulosclerosis, complete effacement of foot processes is seen, whereas in the secondary disease, the foot process effacement is usually segmental.[17] With regard to membranous nephropathy, the diagnosis can be reached most of the time based on hematoxylin-eosin staining and immunofluorescence study results. Electron microscopy is considered helpful in accurate determination of the stage of the disease[17] and excluding the secondary causes, especially in association of lupus nephritis by observing mesangial deposits and tubuloreticular inclusion.

Immunoglobulin M nephropathy was a relatively common entity in this study (13.6%). The light microscopy in most of the cases revealed mild segmental mesangial hypercellularity associated with mesangial matrix examination. The diagnosis can be reached by findings of strong and diffuse mesangial IgM staining.[18] Electron microscopy is not required in these cases, but in some cases, it shows mesangial dense deposits; however, their presence is not required for the diagnosis.

Endocapillary proliferative glomerulonephritis usually presents no diagnostic difficulties when it shows endocapillary proliferation and neutrophilic infiltration in the light microscopy and granular deposits of complement 3 by immunofluorescence study. However, in more chronic cases in which there are less neutrophils infiltration or if the immunofluorescence study is inconclusive, the differential diagnosis from other entities become more difficult. In such cases, ultrastructural demonstration of subepithelial "humps" is needed for the diagnosis of postinfectious glomerulonephritis. In the current study, 7 of 20 cases of postinfectious glomerulonephritis required EM to establish the diagnosis. All of theses cases were in the resolving stage, in which

the differential diagnosis included other entities like lupus nephritis.

Membranoproliferative glomerulonephritis type I and II are difficult to distinguish from each other by light microscopy and immunofluorescence studies. Electron microscopy permits proper subtyping of membranoproliferative lesion. In cases of amyloid nephropathy, positive Congo red stain, immunofluorescence, and immunohistochemistry are sufficient to make the diagnosis. However, in nonamyloid fibrillary glomerulonephritis, examination by EM is considered essential for the diagnosis.

In IgA Nephropathy and Henoch-Schönlein nephritis, light microscopy can show various patterns, ranging from normal, focal and segmental mesangial hypercellularity, and diffuse hypercellularity to sclerosis. The definitive diagnosis of this entity is obtained by immunofluorescence with the detection of IgA in the mesangial areas. Electron microscopy is complementary as it confirms the diagnosis by revealing the electron-dense immune complexes deposits in the mesangial and paramesangial regions. We had one case of IgA nephropathy in which the EM was essential as it demonstrated in addition to IgA, features that were consistent with Alport syndrome. In the group of vascular disease, the diagnosis is normally reached via light microscopy and immunofluorescence and EM are usually not required.

In diabetic nephropathy, thickening of basement membrane may be observed on EM before the clinical signs of diabetic kidney disease.[19]

Lupus nephritis was the most common type of nephropathy in this study. In most cases, clinical history, light microscopy, and full-house pattern of immunoglobulin and complement deposits on immunofluorescence were sufficient to make the diagnosis. Electron microscopy was useful in the cases where the proliferative lesion was mild and focal (Class II versus Class III), in some cases of class V nephritis, in the combined class IV and V, and also in the more chronic cases in which the immunofluorescence findings were less convincing.

We had 2 cases of C1q nephropathy in children with no serological or clinical evidence of systemic lupus erythematosus. Light microscopy showed mesangial proliferative glomerulonephritis, and the immunofluorescence study demonstrated mesangial C1q deposits. Electron microscopy was essential in these two cases by demonstration of mesangial and subendothelial electron-dense deposits and absence of tubuloreticular inclusion.

Kidney transplant biopsies are not included in this study. In a recent study by Collan and coworkers,[15] EM contributed to the final evaluation in 12 of 14 transplant biopsies included in the study (86%), and the contribution was mainly in exclusion of glomerulonephritis and amyloidosis. There is general agreement among renal pathologists that EM is important in the evaluation of renal specimens from transplanted patients with proteinuria in order to distinguish between transplant glomerulopathy and recurrent or de novo glomerulonephritis; however, other indications are still controversial.[20] A relatively recent study by Ivanyi and colleagues evaluated the role of EM in the diagnosis of chronic kidney allograft rejection in a series of 91 transplant biopsies. The results of EM increased the diagnosis of chronic rejection to 69 % of the cases and decreased chronic transplant nephropathy diagnosis to 15%. They strongly recommended the incorporation of EM into the evaluation of late dysfunction transplant biopsies.[21]

CONCLUSIONS

Our study confirmed what had been concluded in the previous studies; although it is possible to diagnose a great percentage of glomerulopathies (61%) based on the light microscopy and immunofluorescence findings alone, EM still has an integral role in the diagnosis of certain entities. If EM cannot be performed routinely in all cases, a small portion of renal tissue should be saved in an appropriate fixative for EM, which could then be performed if needed for any reason.

CONFLICT OF INTERETS

None declared.

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