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RESEARCH ARTICLE

THE AGREEMENT OF LIGHT AND ELECTRON MICROSCOPY IN DIAGNOSIS OF GLOMERULONEPHRITIS DURING 10 YEARS, SOUTHERN IRAN

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ABSTRACT

In variable types of glomerular kidney diseases, evaluation of kidney biopsies by electron, light and IF microscopy is essential for diagnosis confirmation and management planning. Since electron microscopic evaluation of all specimens is not economically cost-beneficial, there should be an evaluation of how necessary is performing it for each type of glomerular disease. We gathered and analyzed pathologic reports from electron and light microscopy of 985 cases of renal biopsies with variable glomerular diseases during years 2000-2010. Analysis of results was done using SPSS software. The p-value < 0.05 considered to significance. We discovered that only in MCD and alport nephrology there is no significant difference between the group in which electron microscopy provided valuable additional information and the group that consisted of cases in which electron microscopy did not add any useful information to light microscopy. Since electron microscopy is an expensive process to be performed routinely for evaluation and diagnosis of kidney diseases, it should be wisely selected for diagnosis of those disorders that need to be evaluated by electron microscopy.

INTRODUCTION

Histological evaluation of kidney biopsies is the gold standard for diagnosis of most renal glomerular diseases. This evaluation can be performed by light microscopy, immunohistochemistry or electron microscopy (Spargo, 1975). Nowadays electron microscopy is being used for confirmation of diagnosis based on light microscopy and immunohistochemistry. As reported that about 85% of kidney biopsies had an indication of electron microscopy for diagnostic information (Tighe and Jones, 1970). But due to overwhelming pressure for cost reduction in health care properties, several studies have been initiated to re-evaluate the necessity of electron microscopy for diagnosis of varieties of glomerular diseases (Haas, 1996; Wagrowska-Danilewicz, 2007). A large study showed in 11% of cases, electron microscopy suggested different diagnosis than primary light microscopy and in an additional 36% ultrastructural studies provide additional information to the findings of light microscopy (Siegel *et al.,* 1973). In similar studies, electron microscopy indicated a different diagnosis than light microscopy alone to 13% of renal biopsies (Olsen *et al.,* 1983; Muehrcke *et al.,* 1969). Some studies showed that that diagnosis of some renal biopsy such as alport syndrome, dense deposit disease, lysosomal storage diseases, diabetic nephropathy, minimal change nephropathy and etc. usually require electron microscopy study (Tighe and Jones, 1970; Haas, 2007). In addition several new glomerular diseases and variants have been described in which ultra structural findings are useful in establishing the diagnosis (Pearson *et al.,* 1994; D'Agati *et al.,* 1989; Fogo *et al.,* 1993; Jennette and Hipp, 1985).

It seems that supporting data for a definite decision about using electron microscopy for diagnosis of glomerular diseases is lacking and in consideration of the fact that electron microscopy remains a costly and time consuming procedure, this study was undertaken to investigate the diagnostic role of electron microscopy study for native renal biopsies also examined by light microscopy.

MATERIALS AND METHODS

This study is conducted as a retrospective descriptive study of 985 cases of kidney biopsies referred to department of pathology of Shiraz University of Medical Sciences in a 10 year period between March 2000 and March 2010. We gathered medical profiles of all kidney biopsies of glomerular diseases. Renal biopsies were obtained by percutaneous

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approach under sonographic guide following fine needle biopsy standards and the samples were prepared for electron microscopy. For light microscopic examinations the specimens were fixed, processed and embedded in paraffin then thin sections were obtained and stained with hematoxylin and eosin, periodic acid Schiff followed by Jone's silver methenamine and Masson trichrom's staining techniques. For electron microscopy the specimens were fixed in 3% buffered glutaraldehyd, poststainedin 1% osmium tetroxide, processed for electron microscopy and embedded in resins. Thin sections were cut on Leica Ultra cutultramicrotome. Sections were stained with toluidine blue and evaluated under light microscope (Leo 906) for detection of glomeruli. If semi-thin sections contained glomeruli, ultrathin sections were obtained from resin blocks, transferred to the copper grids then grids were stained with uranyl acetate and lead citrate and examined with a Leo 906 electron microscope.

All cases of renal biopsies indicating a glomerular disease were included in this study if the complete reports of electron and light microscopy were available. Furthermore the findings were evaluated and the biopsies were assigned to one of the three categories on the basis of how ultra-structural findings contributed to the primary diagnosis; Essential group consists of biopsies in which the findings of electron microscopy were different from light microscopic study and making the correct diagnosis absolutely requires electron microscopy evaluation.
If the electron and light microscopic findings were the same, however, electron microscopy was necessary to provide important information confirming or strengthening the primary diagnosis, the biopsies were included into group Important. In group not required, the electron microscopy was not needed to confirm the diagnosis and did not supply other clinically pertinent information related to diagnosis.

The statistical analysis of data was performed by chi-square test using "Statistical Package for Social Sciences (SPSS) 18.0 for Windows" statistical software to reveal the significance of difference between observed results and assumption of 50% necessity of electron microscopy study. The results related to the continuous variables were presented as mean ± SD. And those related to quantitative or categorical data were shown as percentage or frequency, that analyzed by chi-square test. A p-value less than 0.05 were considered to be statistically significant and the confidence interval was assumed to be 95%.

RESULTS

The present study was conducted on 985 cases of glomerular kidney disease to find out the necessity of EM studies in diagnosis of renal glomerular diseases. The mean age of studied population was 28.3 ± 8.6. Forty one point nine percentages (413 cases) of all cases was male and 58.1% (572 cases) were female. The distribution of cases within the categories of glomerular disease is presented in Table 1.

As shown in Table 1, the highest frequency among all investigated glomerular disorders goes to lupus nephritis (27.0%), followed by membranous GN (20.3%), mesangioproliferative GN (15.6%) and focal-segmental glomerulosclerosis (13.8%). Also, we analyzed the data in separated categories to define the frequency of each agreement group of Essential, Important or Not required in the mentioned disorders (Table 2).

Table 1. Distribution of cases in 985 patients of glomerular disease

Disease	Sample	Frequency (%)
Minimal change disease	87	8.8
Membranous GN[1]	200	20.3
Mesangiocapillary GN	70	7.1
Mesangioproliferative GN	154	15.6
Crescentic GN	23	2.3
Lupus nephritis	266	27.0
Diabetic nephropathy	13	1.3
Focal-segmental GN	136	13.8
Alport nephropathy	6	0.6
Amyloidosis	19	1.9
Hemolytic uremic syndrome	11	1.1
Total	985	100.0

[1]glomerulonephritis

Table 2. Cross tabulation of glomerular disease with degree of agreement between electron and light microscopy

Diagnosis	Agreement (n, %)			Total
	Essential	Important	Not required	
Minimal change disease	44.9% (39)	1.1% (1)	54.0% (47)	100.0% (87)
Membranous GN[1]	11.5% (23)	5.5% (11)	83.0% (166)	100.0% (200)
Mesangiocapillary GN	17.1% (12)	7.1% (5)	75.7% (53)	100.0% (70)
Mesangioproliferative GN	22.7% (35)	1.9% (3)	75.3% (116)	100.0% (154)
Crescentic GN	4.3% (1)	4.3% (1)	91.3% (21)	100.0% (23)
Lupus nephritis	1.1% (3)	3.4% (9)	95.5% (254)	100.0% (266)
Diabetic nephropathy	0.0% (0)	15.4% (2)	84.6% (11)	100.0% (13)
Focal-segmental GN	23.5% (32)	2.2% (3)	74.3% (101)	100.0% (136)
Alport nephropathy	83.3% (5)	0.0% (0)	16.7% (1)	100.0% (6)
Amyloidosis	10.5% (2)	0.0% (0)	89.5% (17)	100.0% (19)
Hemolytic uremic syndrome	9.1% (1)	0.0% (0)	90.9% (10)	100.0% (11)
Total	15.5% (153)	3.6% (35)	80.9% (797)	100.0% (985)

[1]glomerulonephritis

Table 3. Results of statistical analysis to reveal the significance of difference between expected and observed data

Diagnosis	p-value	Expected		Observed	
		Essential+ Important	Not required	Essential+ Important	Not required
Minimal change disease	0.453	43.5	43.5	40	47
Membranous GN[1]	0.000	100	100	34	166
Mesangiocapillary GN	0.000	35	35	17	53
Mesangioproliferative GN	0.000	77	77	38	116
Crescentic GN	0.000	11.5	11.5	2	21
Lupus nephritis	0.000	133	133	12	254
Diabetic nephropathy	0.013	6.5	6.5	2	11
Focal-segmental GN	0.000	68	68	35	101
Alport nephropathy	0.102	3	3	5	1
Amyloidosis	0.001	9.5	9.5	2	17
Hemolyticuremic syndrome	0.007	5.5	5.5	1	10
Total	-	492.5	492.5	188	797

Since frequency is not reliable descriptive measures, chi-square test was performed to find out the significance difference between observed frequencies and expected measures which was set to be equal frequencies in groups Essential + Importantversus group Not required. This assumption would be correct only if there was no difference between using electron microscopy for evaluation of biopsies or not (Table 3).

As shown in Table 3, if we consider p-value < 0.05 to be significant, in all investigated glomerular diseases except MCD and alport nephropathy there is significant difference between what we observed and the assumption that the use of electron microscopy is equivocal. In all disorders except MCD and alport nephropathy, the majority of cases belong to group Not required of agreement which means that the data provided by electron microscopy is not necessary for making the diagnosis and also it does not add any confirmatory or useful information to what light microscopy provides.

DISCUSSION

Electron and light microscopic studies are the goal standard work-ups for diagnosis of glomerular kidney diseases. Deciding to choose between electron or light microscopic studies has been a field for debates since economic matters play important role in choosing the treatment and work-up strategies in medicine (Wagrowska-Danilewicz 2007).

In our study we found that in 19.1% (15.5% essential and 3.5% helpful) of all studies cases, electron microscopic evaluation is valuable for making the diagnosis. A study has previously stated that electron microscopy may have indications in about 85% of cases of glomerular diseases (Tighe and Jones, 1970). Pearson *et al.* found that routine electron microscopy is valuable for diagnosis of certain GN such as membranous GN and MCD (Pearson *et al.,* 1994). Sementilli *et al*. (2004) conducted a study with 200 cases to define the role of electron microscopy for diagnosis of glomerulopathies, they found that electron microscopy was necessary for diagnosis of only 10% of renal biopsies and the diagnosis of 85-90% of cases can be made by light microscopy and IF study. Among the 10% that should be evaluated by electron microscopy, most cases are hereditary glomerulopathies and MCD that correlate well with our findings. Also in 2007, another study of 113 cases was conducted by W'growska-Danilewicz with the same aim and they found that electron microscopy is essential for diagnosis of alport nephropathy and thin basement membrane disease (Wagrowska-Danilewicz 2007). Elhefnawy in a study found that totally in 25% of renal biopsies contributing to 100% of hereditary glomerulopathies and 23.5% of other types of glomerulopathy, electron microscopy provides essential information for diagnosis of the disease (Elhefinawy, 2011).

Another study of 52 renal biopsies was conducted by Darouich in 2010, they had performed electron microscopy for 20 cases out of 52 because diagnosis could not be established properly by light and IF microscopy. Results of electron microscopy revealed that electron microscopy was essential for diagnosis of 8 cases (40%) and helpful in 12 cases (60%), (Darouich *et al.,* 2010). our results are similar to previous studies and are most agreeing with the results of Elhefnawy (2011). In our study the electron microscopy findings were crucial for diagnosis in 15.5% of glomerulopathies cases and the highest percentage of cases in "essential" group was Alport nephropathy and MCD. 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 nappearance (Mazzucco *et al*., 1998). Also, a typical example of kidney disease in which electron microscopy is essential in diagnostics is MCD (Rivera *et al*., 2001). In a similar study by Haas *et al*. (1996) ultrastructural findings were crucial for diagnosis in 21% of cases and the highest percentage in this group goes to thin basement membrane nephropathy and MCD. Also in another study, Mokhtar *et al.* (2011) found that electron microscopy was essential in diagnosis of 17% of cases including MCD, hereditary nephritis, fibrillary GN, and certain classes of lupus nephritis (Mokhtar and Jallalah, 2011). In our 35 cases (3.6%), the electron microscopy findings did not alter the preliminary diagnosis, however did provide important information confirming or strengthening this primary diagnosis.

In Haas study (Haas, 1996) 21% and in Mokhtar study (Mokhtar and Jallalah, 2011) 39% of cases belong to "Important" category which is much greater than our result. The electron microscopy findings were not of any help in establishing the diagnosis and did not obtain any valuable information in 80% cases of renal biopsies. Pearson *et al*. (1994) concluded that only in 25% of cases electron microscopy was unhelpful for diagnosis. Similarly, in study of Collan *et al.* (2005) only about 25% of the electron microscopy reports did not have any influence on diagnosis process.

Conclusion

As it can be noticed our study is the largest series (985 samples) ever conducted for evaluation of the role of electron microscopy for diagnosis of glomerular diseases; as in 19% of glomerulopathies the electron microscopy study provides fundamental or important diagnostic information, and therefore electron microscopy remains a useful tool in the diagnosis of glomerular diseases. Duo to that some categories our cases were lacking the sufficient quantity to produce statistically reliable results, we recommend that further studies should be performed specifically targeted for evaluation of the role of electron microscopy in those diseases.

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