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Comparison between light and electron microscopic findings in 30 patients with lupus nephritis

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Abstract

Background: The kidney biopsy specimen is used for initial diagnosis of patients with SLE who at the time of biopsy lack either diagnostic clinical manifestation and or serological markers. Another role is evaluation of renal dysfunction in transplanted patients when lupus has occurred in renal allograft. The aim of this study is correlating the findings of light, immunofluorescent and electron microscopy in thirty patients with lupus nephritis.

Methods: The kidney biopsies of thirty patients with SLE were studied for purpose of correlating the findings of light, immunofluorescent and electron microscopy. We studied 30 parameters in light microscopy sections, 5 parameters in semi -thin and EM sections , and IgG, IgM, IgA, C3, C4 and fibrinogen in different structures of specimens by immunofluroscent microscopy. The P value and measurement of agreement of kappa was calculated.

Results: In 25 cases LM and EM correlated completely including lupus nephritis class, activity and chronicity indices and presence or absence of immune complex deposition. In 5 cases discrepancy between Light Microscopy and Electron Microscopy diagnosis was found. Three cases were classified as class III according to LM and class II by EM. LM reevaluation of all three cases showed focal and segmental endocapillary cell proliferation with neutrophilic infiltration. We found that LM study is cornerstone in the focal lesions because of the limited inclusion of glomeruli in EM. One case of class IV by LM, in EM shows massive (grade III) sub-epithelial depositions and grade I sub endothelial deposition and was classified it as Class V + VI. In LM, findings cellular crescent in six glomeruli, severe endocapillary cell proliferation with activity index of (16/24) were detected. So the correct diagnosis was Class V + VI. The last case classified as IV in LM classification and revealed moderate mesangial cell proliferation with obliteration of lumens. In EM, we had three glomeruli which all showed mesangial cell proliferation, grade II mesangial deposition, with one focus of small (grade I) sub endothelial deposition. According to the above-mentioned findings the EM class of patient was class II.

Conclusion: We found that there is agreement between EM and semi-thin sections for detection of exact site of depositions as well as their grading. Study of semi-thin sections by LM can demonstrate the deposits that are observed on EM.

Keywords: Lupus nephritis; Electron microscopy; Light microscopy

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Introduction

Systemic lupus erythematosus is a kind of dysregulation of the immune system along

with the production of auto-antibodies and resulting immune complex reaction.[1] The diagnosis of SLE requires correlation of clinical and laboratory features. Lupus nephritis is one of the most significant manifestations in SLE and the course of SLE depends on the type and severity of renal involvement. Renal biopsy specimens serve as a good indicator of natural history and response to the treatment.[1]

Determination of the immunomorphologic characteristics, pattern and distribution of renal involvement is important and critical for better evaluation of patient's status. Renal biopsy therefore can be extremely valuable in the management of patients with lupus nephritis.[2]

The role of triple study including light (LM), immunofluroscence (IF) and electron microscopy (EM) in diagnosis and classification of lupus glomerulonephritis should not be underestimated and may even be essential in some cases. The lack of readily available EM facilities in some centers, and its high cost was to evaluate the role of EM study for exact diagnosis and better evaluation of lupus nephritis given the fact that EM may not be available readily in some centers on account of its high cost. We studied 30 renal needle biopsies from patients with lupus nephritis to find correlation between LM, IF and EM finding in lupus nephritis.

Materials and Methods

During a 45-month period in 2000–04, thirty consecutive kidney biopsy specimens from clinically suspected lupus nephritis patients (adult and children) were selected from the affiliated hospitals of Shiraz University of Medical Sciences. The mean age of patients in this study was 25.5 (range: 9–47) years with a female to male ratio of 6.5/1. We studied only those with clinical diagnosis of SLE which had specimens for triple study including LM, IF and EM with following criteria:

1) LM: Adequate renal cortical tissue with at least ten glomeruli after fixation of needle biopsy in 10% formalin from paraffin embedded blocks were prepared, in multiple thin sections (2-3 micrometers) and stained with H&E, silver, Masson trichrome and PAS stains. Finally, we studied at least five slides in each case comprising two H&E, one silver, one Masson and one with PAS stain.

2) IF: Adequate renal cortical tissue which were snap-frozen for direct immunofluorescent were cut as thin as possible (2-4 micron) and stained with antisera known to be relatively monospecific for IgG, IgM, IgA, C3, C4 and fibrinogen and then searched for depositions in glomeruli, tubular basement membrane, interstitium and blood vessels.

3) EM: Adequate renal tissues which were in glutaraldehyde with at least two glomeruli were studied for EM and all cases were also assessed with semi-thin sections stained with toluidine blue.

4) Relevant data including sex, age, clinical presentation, blood pressure and para clinical information were also recorded.

We evaluated each case in a blind manner. First, we studied the LM slides with adequate number of glomeruli, and a checklist was completed with 30 parameters as listed below:

1- number of glomeruli, 2- lupus nephritis class according to WHO and ISN/RPS, 3- crescent formation (cellular, fibrocellular and fibrous), 4- necrosis, 5- karyorrhexis, 6- PMN leukocyte infiltration, 7- endocapillary cell proliferation, 8- subendothelial deposition, 9 and 10- hyaline thrombi and wire loop, 11- fibrin thrombi, 12- segmental or global sclerosis, 13- GBM thickening, 14- capillary lumen

Table 1: Prevalence of different classes of lupus nephritis (WHO Classification)

Class	n (%)
I	0 (0)
II	4 (13.3)
III	5 (16.6)
IV	18 (60)
V	2 (6.6)
VI	0 (0)
V + IV	1 (3.3)

Table 2: Prevalence of different activity grades in 30 patients with lupus nephritis

Activity grade	N (%)
I (0-8)	12 (40)
II (9-16)	16 (53.3)
III (17-24)	2 (6.6)

status, 15- tubular atrophy, 16- tubular basement membrane deposition, 17- tubular necrosis, 18- tubular cast, 19- interstitial inflammation, 20- interstitial fibrosis, 21- interstitial deposition, 22- vasculitis, 23- vessel wall necrosis, 24- vascular intimal thickening, 25- vascular medial hyperplasia, 26- vascular hyaline thickening of the wall, 27- vascular wall deposition, 28- vascular wall sclerosis, 29 and 30- the activity and chronicity indices according to the Austin scoring system.[1] For IF study all data regarding positivity of IgG, IgA, IgM, C3, C4 and fibrinogen and also intensity of staining as trace, grade I, II, III in four different structures of specimen were evaluated. For EM study we reviewed first the entire semi-thin sections and then the grids for presence of immune-complex depositions in sub epithelial, intramembranous, sub-endothelial, and mesangial, TBM, interstitium and Bowman's capsule. We graded these depositions as 1-3 (minimum, moderate and massive) in accordance with their number and extensions. Grade 1 was defined as one or two very small deposits, grade 2 as several small and large non-fused deposits and grade 3 as abundant amount of fused deposits. Most of them occupied a large part of the entire circumference of a capillary loop or most of the mesangial matrix.[3] Finally, we analyzed all data from different steps (LM, IF, semi-thin sections and EM).

We used the kappa coefficient for this purpose for evaluation of LM and EM study. No statistical method for evaluation of this agreement for IF data is present because we can not classify lupus nephritis according to the IF pattern only, and IF was used for confirmation of diagnosis.

Results

The glomerular number in light microscopic slides was from 10 to 32 (mean: 17.03). Weening, believed that the acceptable number is ten for LM, one for IF and one for EM study.[4] The prevalence of different class of lupus nephritis in our study is summarized in Table 1. The prevalence of various classes of lupus nephritis in different studies is as follow:

Class II (20%), class III (20%), class IV (40%), class V (15%) and class I and VI (5%) respectively[2,5] which are close to our results.

The activity index was calculated according to the Austin, et al. scoring system on LM slides.[1] We divided the wide range of activity (0-24) into three groups, I (mild activity), when the activity index was between zero to eight, II (moderate activity), when it was between nine to sixteen, and III (severe activity), when between seventeen to twenty four.

The prevalence of activity and chronicity grading and scoring indices in our study are

Table 3: Prevalence of wire loop and hyaline thrombi in proliferative lupus nephritis (Class III, IV and IV + V)

Class	Wire loop n (%)	Hyaline thrombi n (%)
III	4 (80)	3 (60)
IV and IV + V	16 (84)	12 (63)

summarized in Table 2. All our cases had a chronicity index below four based on Austin et al. scoring system, which is from zero to twelve.[1] Two cases (6.6%) showed necrotizing vasculopathy (lupus vasculopathy) with marked deposition of immune complexes in vessel walls. The reported percentage of this vascular lesion in lupus nephritis is 10%.[4] Three cases show uncomplicated vascular immune complex deposition (10%) with activity indices of 17/24, 9/24 and 11/24, respectively.

Jennete, *et al.*, claim that uncomplicated vascular immune deposition is the most common vascular lesion and occurs in approximately 17% of patients with lupus nephritis.[5] We observed other vascular changes such as medial hypertrophy and intimal thickening in four patients. The prevalence of the presence of wire loop and hyaline thrombi formation, which indicate sub-endothelial form of immune deposits large enough to be detected by H & E and mostly in proliferative lesions such as class III&IV patients is summarized in Table 3. Herrera et al. believed that presence of wire loop in LM study is correlated with massive sub enothelilal deposition in EM.[2, 5]

In our study 15 out of 16 patients with class IV lupus nephritis demonstrated wire loop formation in LM and grade III subendothelial deposition in EM (94%). No fibrin thrombi were identified. Twenty-seven cases had positive IF study (90%). The negative results belonged to class II (2 patients) and class IV (one patient), all of whom had mild activity indices. Positive staining for IgG was found in more than 90%, IgM and IgA in about 60% and 70% of the cases, respectively. C3 was the most common complement component and found in approximately 80% of cases. C4 is less common and stains less intensely.[1]

All cases with positive IF showed granular deposition. No linear deposition described as an unusual variant of tubulo-interstitial nephritis in lupus nephritis, and mediated by anti-tubular basement membrane antibodies was recognized.[5]

In our study, lupus classification and all-important criteria in 25 patients was almost identical for both LM and EM study (83%). There was discrepancy between LM and EM findings in five cases (16.7%). Three cases were classified as class III according to LM and class II by EM. Reevaluation of all three cases showed segmental proliferation with neutrophilic infiltration in less than 50 % of the glomeruli but due to the presence of mesangial deposition and absence of sub endothelial ones they were classified as class II in EM which harbors only one glomerulus in each case, indicating that LM diagnosis was correct.

One case of class IV by LM, show massive (grade III) sub epithelial depositions in EM and grade I sub endothelial deposition were classified as Class V according to EM findings. In LM finding six cellular crescents (6/15), with severe endocapillary cell proliferation and 16/24 activity index was detected. In poorly prepared silver stain obvious membranous pattern was seen with a few sub epithelial depositions in 15 evaluated glomeruli. The correct diagnosis was therefore

Table 4: Discrepancy between LM and EM in five cases of lupus nephritis

Case	LM	EM	Final Diagnosis
1	III	II	III
2	III	II	III
3	III	II	III
4	IV	II	II
5	IV	IV + V	IV + V

Class V + VI. Figures 1 and 2 show semi-thin section and EM of this case respectively.

The last case in LM classification was classified as class IV. LM revealed moderate mesangial cell proliferation with obliteration of lumens. There was no evidence of wire loop, hyaline thrombi, crescent formation, glomerular tuft necrosis, PMN leukocytes or karyorrhexis. Masson-trichrome slides showed few suspicious sub-endothelial deposition and GBM thickening. The activity index was 6/24. In EM, we had three glomeruli which all showed mesangial cell proliferation, grade II mesangial deposition, with one focus of small (grade I) sub-endothelial deposition. According to the above-mentioned findings, the EM class of patient was class II (Table 4). According to newly proposed classification of ISN /RPS, mesangial cell proliferation of all degrees puts the case of lupus nephritis in class II. This discrepancy is a weak point of WHO classification where only mild to moderate mesangial cells proliferation is regarded as class II.

In our statistical analysis, we used the coefficient of kappa was calculated as 0.7 ($p<0.001$). In our study we found TBM depositions in five cases, all of which belonged to class IV lupus nephritis with moderate activity indices. We also used the kappa coefficient for evaluation of agreement between activity (LM finding) and immune complex deposi-

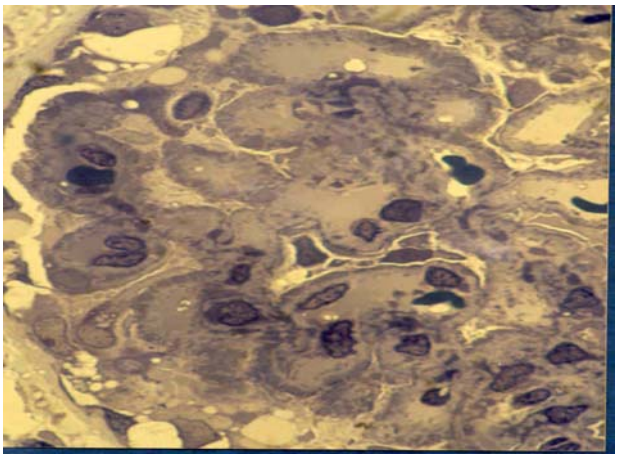

Figure 1: Lupus nephritis, class V and IV, semi-thin section with mostly subepithelial deposition (×400).

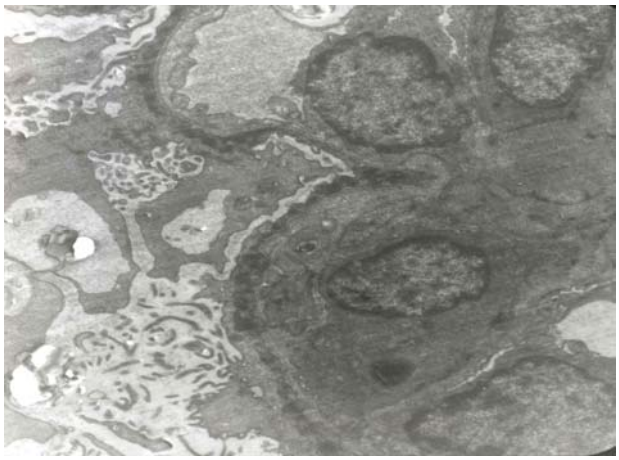

Figure 2: EM, lupus nephritis class V and VI, uranyl acetate lead citrate, subepithelial deposition (×4646).

tion (EM), for proliferative classes of lupus nephritis (class III, IV), it was 0.9 ($p<0.001$). But, we do not obtain a meaningful relationship between activity "severity" and grade of deposition (kappa coefficient was <0.4).

Herrera believes that ultra-structural evaluation does not play a direct role in the determination of these indices. Its role is mainly complementary, but EM does provide a good estimate of activity based on the number and distribution of immune complexes with sub endothelial deposits representing the most important factor to be evaluated.[2]

We had two cases with mild activity index that showed grade III sub enodthelial deposition in EM. Thus, reporting of activity index and grade of deposition in final pathologic report for patient's management is advised. In our study, no fingerprint type immune complex deposition or tubuloreticular inclusions in endothelial cells were identified. These findings are highly suggestive of lupus nephritis. There are valuable data that we recommended to be included by pathologist in EM report.

We studied the semi-thin sections and evaluated them for sub-epithelial, intramembranous, sub endothelial and mesangial deposition and graded similar to EM deposits.

Discussion

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease of which etiology and pathogenesis are not completely understood. Lupus nephritis is one of the most significant manifestations of SLE with significant mortality and morbidity. The morphologic changes in renal biopsy from a patient with SLE comprise a spectrum of vascular, glomerular and tubulointerstitial lesions.[4] In some cases there is no correlation among clinical, serologic and histologic findings. Renal biopsy and histopathologic classification with determination of the activity and chronicity of lupus nephritis are considered necessary before treatment.[2] Determination of the immuunomorphologic characteristics, pattern, and distribution of renal involvement is important for clinical management.

Correlating LM, IF and EM findings within the clinical context of lupus nephritis cases is crucial for appropriate clinical management.[6] In a clear subset of these patients with lupus nephritis, EM plays a pivotal role in accurately characterizing the type of renal involvement and determining the degree of activity, providing useful and objective guides for patient's management & treatment. Ultra structural evaluation can also be crucial in the initial diagnosis of patient with lupus who, at the time of biopsy, lacks either diagnostic clinical manifestations or serologic markers, and are, therefore clinically unsuspected. EM evaluation also plays a significant role in the evaluation of renal dysfunction in transplant patients, helping to determine whether recurrence of the lupus has occurred in the renal allograft.[2] Some ultra-structural findings, in the proper clinicopathologic context are very suggestive or even diagnostic of lupus nephritis.

Guuillermo, *et al.*, believe that EM will be found necessary for a more precise classification of lupus nephritis and this method will continue to be used.[2]

Hass in a large study studied 213 renal biopsies and found that EM was needed for a correct diagnosis in 11% of cases, as well as for confirmation or additional information in another 36%. They said that EM findings in lupus nephritis are confirmatory, provide additional information and confirmation.[7] Herrera, claimed that EM is necessary in some

cases for initial diagnosis, determination of the immunomorphologic characteristics, pattern and distribution of renal involvement which is important for clinical management. Correlating LM, IF and EM findings within the clinical context of lupus nephritis is crucial.[10] Ferluga et al. reported that class determination has a role for determining further treatment, and conclude that LM, IF, EM studies are valuable.[11] Su et al. demonstrated that detection of finger print deposits in lupus nephritis usually show subsequent development of diffuse type of lupus nephritis.[12] Weening, et al. said that the role of EM in the diagnosis and classification of lupus glomerulonephritis can not be underestimated and may be essential in some cases. The lack of readily available EM facilities in many centers throughout the world should not prevent the skilled pathologist from rendering a diagnosis of lupus nephritis using a combination of complete LM and IF studies.[4] MC-Cune et al. suggested that the EM stage especially in advanced membranous nephritis (class V) provided important information regarding the extent of renal injury and they are associated with worse renal function in patients' comparable WHO classification and NIH activity and chronicity indexes without advanced membranous type.[13] Kraft said that the single most common morphologic features associated with nephrotic proteinuria is diffuse visceral epithelial cell foot process effacement. They concluded that the development of nephrotic range proteinuria in patients with SLE and without peripheral immune aggregate deposition or endocapillary proliferation is more likely a manifestation of SLE than the coexistence of idiopathic minimal change disease and SLE.[14] Haya-Kawa et al. believed that EM study in lupus nephritis disclosed the deposition of immune complex in TBM, the capillary wall and the interstitum proper.[15]

In 25 cases (83.3%), our LM and EM findings were identical with the same classification, type of lesions, activity and chronicity status. In five cases (16.7%) there was discrepancy between LM and EM results.

Electron Microscopy in three patients which were classified as class III with LM, we found that due to the presence of segmental active and proliferative lesions in less than 50% of the glomeruli and only mild mesangial deposition with no sub-endothelial ones, they better be classified them as class II. In all three cases only one glomerulus was identified, so, this discrepancy was due to the limited number of glomeruli in EM and adequate ones in LM, hence LM study was diagnostic in these three patients (60%).

One case, with LM diagnosis of class IV which showed moderate endocapillary cell proliferation, suspicious focal area of sub endothelial deposition and karyorrhexis was classified as class II by EM due to presence of only grade II mesangial deposition. No wire loop, hyaline thrombi, PMN leukocyte infiltration was seen in LM. According to the newly proposed classification of (ISN/RPS, 2003) classification of lupus nephritis any degree of mesangial cell proliferation with no evidence of active and necrotizing lesions can be regarded as class II.[4,8] WHO classification of lupus nephritis (1982) puts only those cases in class II based on mild to moderate mesangial cell proliferation, however, the correct diagnosis was class II and with EM studies (20%).[9]

One case, which was classified as class IV by LM, showed grade III, diffuse sub- epithelial depositions as well as grade I-II diffuse sub endothelial ones in EM and was classified

as Class IV + V .We reviewed the new silver stained sections and found diffuse sub-epithelial deposition, in>50% of glomeruli which encircled more than half of the glomerulus. Poor technique of first silver stain was the cause of difficulty of sub epithelial recognition. We reached the correct diagnosis in EM study (20%). In comparing the LM and EM classification of lupus nephritis we obtained a significant Kappa coefficient: 0.77 ($p<0.001$).

We realized that EM has limitations in evaluation of focal lesions. Light microscopic study of good biopsies with adequate number of glomeruli, proper light microscopic sections (at least five slides stained with H & E, PAS, Silver and Masson Trichrom), evaluated by skilled nephropathologist and use of new classification of lupus nephritis can provide exact classification and determination of activity and chronicity of the lesions and achieved critical data necessary for patients management and therapy in most of the cases. Since EM technique is not available everywhere, is expensive and a non rapid method we recommended taking biopsy for EM study with appropriate fixation and storage of a sample with renal cortical tissue for ultra structural evaluation and use it when it is needed.

We also found that there is agreement between EM and semi-thin sections for detection of exact site of depositions as well as their grading with kappa coefficient =0.9 ($p<0.001$).

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