Original Article

DOI: 10.21276/APALM.1889

Native Renal Biopsy: An Essential Diagnostic Tool in Systemic Lupus Erythematosus

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a connective-tissue disorder of autoimmune aetiology and presented with broad range clinical manifestation due to multisystem involvement. In spite of overall reduction in morbidity due to recent therapy, renal involvement is the leading cause of disease related mortality.

Materials and Method: We conducted a cross-sectional observational study to assess clinicopathological findings and to identify the prognostic association of histopathological parameters with advanced clinical stage. We included 31 patients met the diagnostic criteria of SLE according to revised criteria of the American College of Rheumatology (ACR) for SLE in 1997. Each native renal biopsy was examined by two trained pathologists by light microscopy and was classified using ISN/RPS 2003 lupus nephritis classification system.The Kruskal–Wallis test was performed for comparisons between multiple groups.

Result: Pedal oedema were found to be the most common clinical presentations.. Female preponderance is noted in present study with male: female ratio 1:9.3. We found diffuse proliferative glomerulonephritis (class IV) as most frequent class with incidence rate of 54.8%. After combination of the variables along with different classes of lupus nephritis, significant statistical association was observed in endocapillary proliferation and neutrophillic in filtration as activity predicting factors. Silent LN has been observed in class II as well as in class IV disease alsoThe most common deposited immunoglobulin was IgG.

Conclusion: Renal biopsy remains the main diagnostic tool in identification of exact stage of involvement because clinical staging may not accurately corroborate with histopathological staging.

Keywords: Systemic Lupus Erythematosus, Native Renal Biopsy, Light Microscopy, Immunofluorescence.

Introduction

Systemic lupus erythematosus (SLE) is a connective-tissue disorder of autoimmune aetiology and presented with broad range clinical manifestation due to multisystem involvement. In spite of overall reduction in morbidity due to recent therapy, renal involvement is the leading cause of disease related mortality. Depending on the study population, 25% to 75% patients with SLE presented with lupus nephritis.[1,2] Asian population reveals a higher incidence rate ranging from 33% to 55%. [3,4] The interval of initial diagnosis and emergence of renal impairment varies from 6 to 36 months.[5] Proteinuria followed by haematuria and renal failure are the presenting features depending on the stage of kidney involvement.

Varied range of morphological changes are noted starting from podocytopathy to global sclerosis. Exact diagnosis is essential for individual categorical management. Renal biopsy remains the main diagnostic tool in identification of exact stage of involvement because clinical staging may not accurately corroborate with histopathological staging. Silent LN has been observed in class II as well as in class IV disease also.[6] New ISN/RPS classification provides detailed definitions of specific categories along with activity and chronicity index. Initiation of more aggressive immunosuppression is essential in presence of high activity index and on the contradictory avoidance of overtreatment in presence of chronic lesion.

We performed our present study with patients of lupus nephritis regardless of clinical category. Histopathological findings were compared with clinical presentation and prognostic association of different light microscopic parameters were assessed.

Material and Methods

We conducted a cross-sectional observational study between December 2016 to July 2017 to assess clinicopathological findings and to identify the prognostic association of histopathological parameters with advanced clinical stage. We included native renal biopsies from 31 patients met the diagnostic criteria of SLE according to revised criteria of the American College of Rheumatology (ACR) for SLE in 1997. The research was approved by institutional ethical committee

Data Collection: Clinical parameters like age ,sex and symptoms according to criteria of American College of Rheumatology (malar rash, discoid rash, photosensitivity, arthralgia, oral ulcer, edema, neurologic signs, gross hematuria) were documented. Depending on the laboratory parameters and serological finding all patients were categorised into four clinical syndromes.

1. **Occult Nephritis:** hematuria and (or) mild proteinuria (0.5 g/d< quantitative urinary protein <1 g/d), or proteinuria (1 g/d≤ quantitative urinary protein ≤3.5 g/d without hematuria and eGFR≥60 mL/min/1.73 m2
2. **Nephritic Syndrome:** Mild to moderate proteinuria (1 g/d≤ urinary protein excretion ≤3.5 g/d) with hematuria, urinary tube may be accompanied by edema and hypertension and eGFR value of ≥60 mL/min/1.73 m2
3. **Nephrotic Syndrome:** High proteinuria (urinary protein quantitation >3.5 g/d), low serum albumin (<30 g/L), hyperlipidemia, high degree of edema and eGFR≥60 mL/min/1.73 m2
4. **Renal Failure***:* Decrease in glomerular filtration rate (eGFR<60 mL/min/1.73 m2), may be accompanied by anemia, hypertension and edema.

Study Settings: Each native renal biopsy was examined by two trained pathologists by light microscopy and was classified using ISN/RPS 2003 lupus nephritis classification system. Hematoxylin and Eosin (H&E) stain, Periodic Acid-Schiff (PAS) stain, silver methamine stain and Masson's trichrome stain were performed for light microscopy.

Table 1. The 2003 ISN/RPS classification of LN

Class I: Minimal mesangial LN

Class II: Mesangial proliferative LN

Class III: Focal LN (<50% of glomeruli)

Class IV: Diffuse LN (> 50% of glomeruli)

Class V : Membranous LN

Class VI: Advanced sclerotic LN (90% of glomeruli globally sclerosed without residual activity)

For assessing activity and chronicity index, the following parameters were evaluated- 1) endocapillary proliferation,2) cellular crescents, 3) fibrinoid necrosis and karyorrhexis, 4) neutrophillic infiltration, 5) wire loop and hyaline thrombi, 6) interstitial inflammation, 7) fibrous crescent, 8) glomerular sclerosis, 9) interstitial fibrosis and 10) tubular atrophy. Specimen for immunofluorescence microscopy was received in Michelle's medium and was stained using fluorescein isothiocyanate (FITC)-conjugated polyclonal rabbit anti-sera against human IgG, IgM, IgA, complement C3, C1q and fibrinogen (Dako Denmark A/S , DK- 2600). The slides were examined under immunofluorescence microscope(reflected LED fluorescence attachment for OLYMPUS CX41 microscope) was categorised from (+) to (+++). Control slides were examined simultaneously.

Statistical Analysis: The Kruskal–Wallis test was performed for comparisons between multiple groups. The $\chi 2$ test was analysed for categorical evaluation. Correlations were evaluated using Spearman's rank correlation. $p<0.05$ was considered as significant. Statistical software(GRAPHPAD PRISM 5) was used for analysis.

Result

Total 31 patients were included in the present study over the period of eight months. All the patients were diagnosed according to the criteria of the American College of Rheumatology. Female preponderance is noted with male: female ratio 1:9.3. Out of 31 cases, 3 male patients clinically manifested with renal disease and diagnosed histologically as class III and class IV diseases . The average age of male patients was 42.6years (range 40-48 years) and the female patient was 30.1 years (range 15 years to 52 years) in this study. There was no significant correlation between the patient age and histological stage in renal biopsy(p= 0.2854).

Clinical Manifestation: The most common clinical presentation was nephrotic syndrome followed by nephritic syndrome, occult nephritis and advanced renal damage. Twenty five patients out of 31 presented with pedal edema(80.6%). All patients had photosensitivity, oral ulcer, alopecia, arthritis, serositis and pulmonary manifestation(100%). We found independent association of age , range of proteinuria, prevalence of hypertension along with class IV of lupus nephritis. Significantly decreased eGFR ($p = 0.04$) was observed in lupus class III and class IV disease. On the contradictory silent LN has been observed in class III as well as in class IV disease. Those patients presented only with subnephrotic proteinuria.

Histopathology: For Lupus Nephritis classification we followed ISN/RPS 2003 classification. We found 2 cases LN in class I(figure 2), 8 cases in classII(figure 3), 2 cases in class III (figure 4),17cases in class IV (figure 5),1 case of combine class IV and V and A single case in classV (figure 6).Activity and chronicity index were also done in class III,IV and combined class IV+ V cases. We got intraglomerular endocapillary proliferation in all class III,classIVand combined class IV+V cases.

It was completely absent in class I, classII and class V.Neutrophillic infiltration was seen in 2 cases of class IV LN(11.7%) and single class IV+V LN case(100%).Wire loop lesions was present in 7 classIV LN cases(41.1%) .Intraglomerular necrosis and karyorrhexis was found in single classIV LN cases(5.9%).Cellular cresents was observed in 3 class IV LN cases(17.6%) and single class IV+V LN case(100%). Inerstitial inflammation was found in all the class I, classIII and classIV+V and classV LN cases.4 class II LN cases(50%) and 15 class IV LN cases(80%). Glomerular sclerosis was found in 2 class II LN cases(25%)and 9 classIV LN cases(52.9%). Fibrous crescent was present in 2 class IV LN cases(11.7%). Tubular atrophy was present in all class I LN cases , class III ,class IV+V and class V LN cases(100%). 4 class II LN case(50%) and 15 classIV LN cases(80%) showed tubular atrophy. After combination of the variables along with different classes of lupus nephritis, significant statistical association was observed in endocapillary proliferation and neutrophillic in filtration as activity predicting factors. Among the chronicity index, tubular atrophy and interstitial fibrosis showed good clinicopathological correlation.

Immunofluorescence Finding: Out of 31 patients 26 showed IgG deposits(86.5%) in Immunofluorescence study , 23 cases showed IgM deposits(75.4%), 20 out of 31 showed IgA(65.6%) and 25 out of 31 showed C3 deposits(80.1%). The most common deposited immunoglobulin was IgG.

Table 1: Correlation of clinical parameters with histopathological stages.

	I	II	III	IV	IV+V	V	P VALUE
No	6.4%	25.8%	6.4%	54.8%	3.2%	3.2%	
Mean age	35	34	41	30.11	15	20	0.2854
Male	0	0	1	2	0	0	0.4049
Female	2	8	1	15	1	1	
Mean Serum creatinine	0.6	0.53	0.42	1.753	0.8	1.2	0.4048
eGFR(<60)	0%	0%	50%	5.9%	0%	0%	0.04*
Hypertension	0%	25%	0%	23.5%	0%	0%	0.3160

Table 2: Prognostic Value of histological variables In Lupus Nephritis (Kruskal-Wallis).

	Class I	Class II	Class III	Class IV	Class IV+V	Class V	P value
Endocapillary proliferation	0%	0%	100%	100%	100%	0%	<0.0001*
Neutrophillic infiltration	0%	0%	0%	11.7%	100%	0%	0.0002*
Wire loop lesion	0%	0%	0%	41.1%	0%	0%	0.829
Fibrinoid necrosis or karyorrhexis	0%	0%	0%	5.9%	0%	0%	0.5577
Cellular crescents	0%	0%	0%	17.6%	100%	0%	0.0906
Interstitial inflammation	100%	50%	100%	80%	100%	100%	0.1277
Glomerular sclerosis	0%	25%	0%	52.9%	0%	0%	0.1669
Fibrous crescents	0%	0%	0%	11.7%	0%	0%	0.3991
Tubular atrophy	100%	50%	100%	100%	100%	100%	0.0261*
Interstitial fibrosis	0%	12.5%	50%	100%	100%	0%	0.0212*

Table 3: Correlation of present study findings with previous studies.

ISN CLASS	Karki et al	Kafle et al	Sobha et al	Gomaa et al	Dhakal et al	Present study
Class I	0	2.5%	0	0	13.5%	6.4%
Class II	5.3%	10%	28.1%	12.8%	35.5%	25.8%
Class III	5.3%	12.5%	21.9%	8.8%	24.3%	6.4%
Class IV	52.5%	52.5%	40.6%	51.4%	18.9%	54.8%
Class IV & V	21.1%	5%	0	0	0	3.2%
Class V	15.8%	2.5%	9.4%	23%	5.4%	3.2%
Class VI	0	2.5%	0	4%	2.7%	0

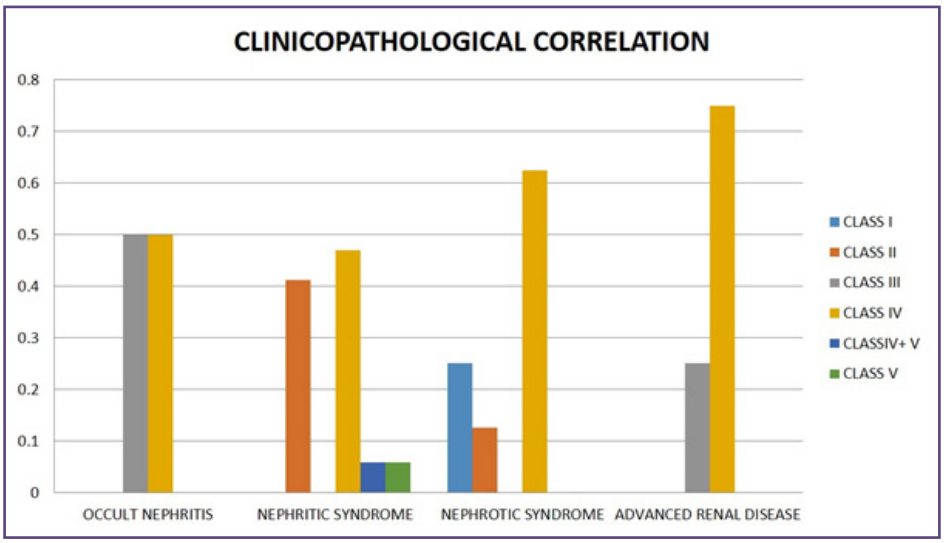

Fig. 1: clinical presentation in different ISN Lupus Nephritis class.

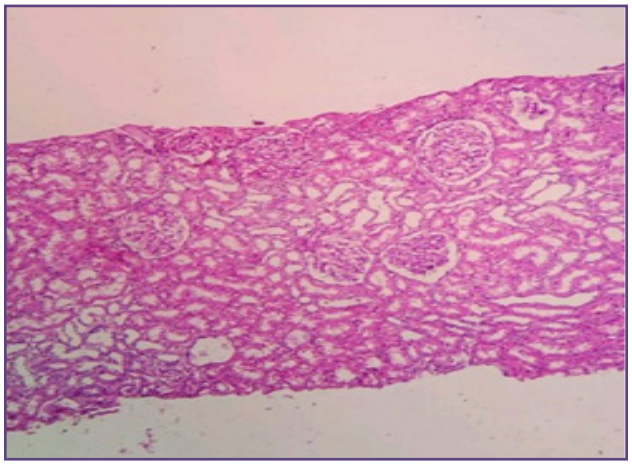

Fig. 2: showing six glomeruli with minimal mesengial changes (H&E,100X).

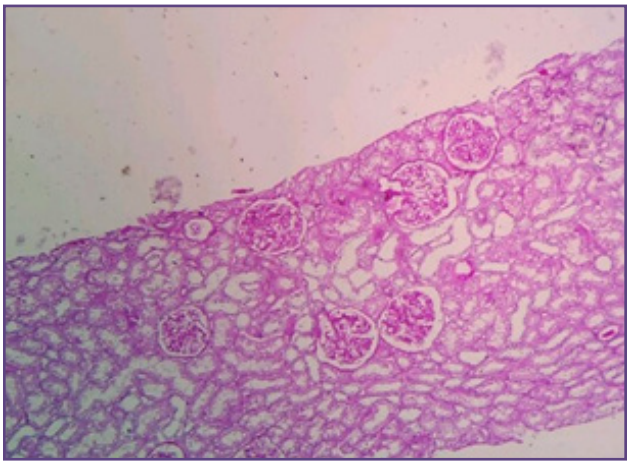

Fig. 3: showing eight glomeruli with mesengial matrix expansion (H&E,100X).

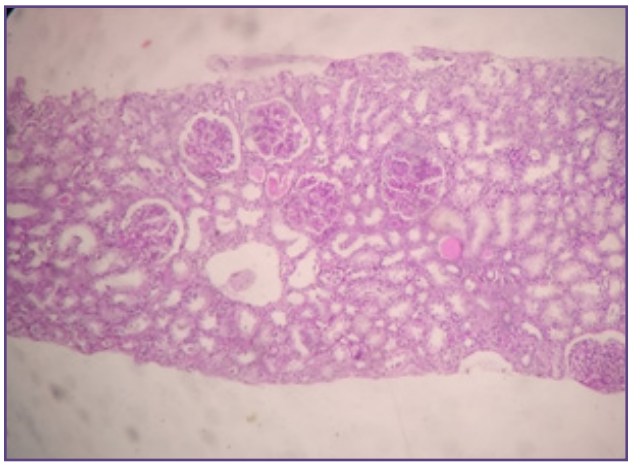

Fig. 4: showing six glomeruli with focal endocapillary proliferation (H&E,100X).

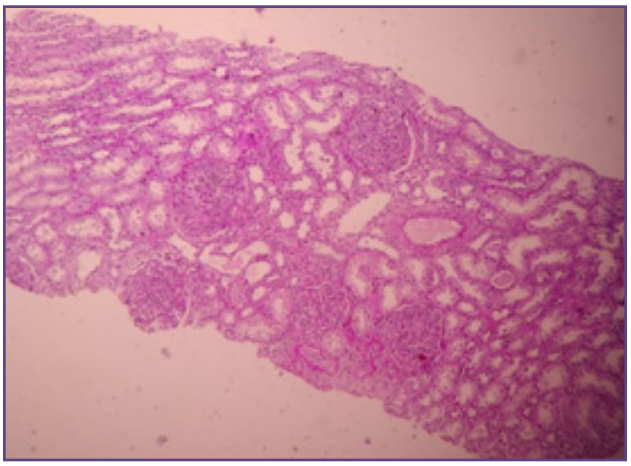

Fig. 5: showing five glomeruli with diffuse endocapillary proliferation (H&E,100X).

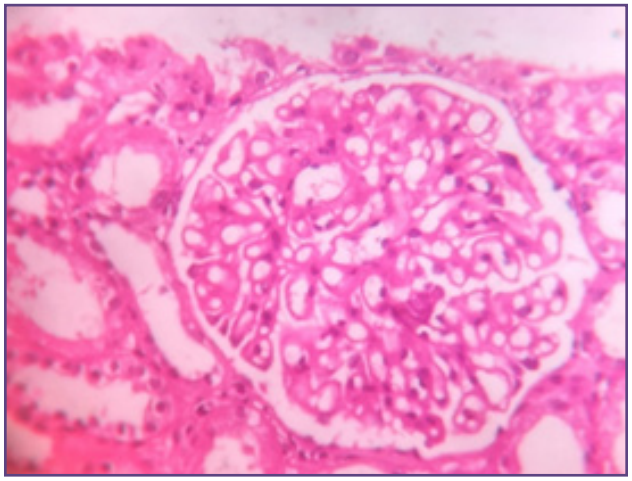

Fig. 6: showing one glomerulus with membranous lupus nephritis (H&E,400X).

Discussion

Present cross-sectional study was carried out in an effort to demonstrate the clinic-pathological correlation in lupus nephritis in our tertiary care institute. Depending on geographical variation, 40%-75% patients of SLE present with renal involvement.[2] Kidney involvement in SLE is a poor prognostic marker as per as the morbidity and mortality concerned.[7] Native renal biopsy is the most essential diagnostic tool for risk stratification for end stage renal disease and also for early intervention to minimize further progression.

SLE can occur in any age group and many studies revealed strong association between clinical manifestation and progression along with age. at any age, and previous reports have demonstrated that age at onset was associated with clinical presentations and outcome.[8,9] But regarding histological staging, relation with age is underdetermined. There was no significant correlation between the patient age and histological stage in renal biopsy(p= 0.2854) in our study. Previous studies documented greater frequency and severity of lupus nephritis in paediatric age group than adult.[10,11] We found only one case of paediatric lupus nephritis aged 15 years and in class IV+V which corroborate with their inference.

Patients sex is an independent risk factor in SLE. Female preponderance is noted in present study with male: female ratio 1:9.3. One study conducted in Iran[12] showed similar findings but another study carried out in Singapore[13] revealed difference. So, geographical location and racial variation are important determinant of clinical presentation.[14,15]In our study 66% male patient reported with oliguria and elevated serum creatinine at the time of kidney biopsy. This observation is supported by the association of estrogen receptor gene polymorphisms with higher susceptibility for lupus nephritis in men.[16]

For Lupus Nephritis classification we followed ISN/RPS 2003 classification. We found diffuse proliferative glomerulonephritis (class IV) as most frequent class with incidence rate of 54.8%. Several previous literature documented class IV as commonest histopathological finding.[17,18,19,20] Significantly decreased eGFR (p = 0.04) was observed in lupus class III and class IV than class I,II,V disease in present study. One previous study finding corroborate with us.[21] On the contradictory silent LN has been observed in class III as well as in class IV disease. Those patients presented only with subnephrotic proteinuria. More exhaustive longitudinal study is required to evaluate the histopathological findings in occult nephritis. Among the clinical parameters, pedal edema showed highest association(p=0.03) with ISN/RPS classes of lupus nephritis. We analyse the variables of activity and chronicity index as independent risk factors. After combination of the variables along with different classes of lupus nephritis, significant statistical association was observed in endocapillary proliferation and neutrophillic in filtration as activity predicting factors. Among the chronicity index, tubular atrophy and interstitial fibrosis showed good clinicopathological correlation.(Table 2). On previous study on childhood lupus nephritis described glomerulosclerosis and tubular atrophy as predictive factors for progression.[22] But in our study we did not reported any case of class VI lupus nephritis.

All cases showed glomerular deposit in our study among which 75% reported as full-house pattern. IgG was the commonest immunoglobulin followed by C3. This immunofluorescence finding is considerably similar with other studies.[23,24]

Conclusion

In lupus nephritis, ISN/RPS class along with activity and chronicity index may be useful as prognostic factors for further progression and determinant of individualised optimal management independent of clinical presentation.

Limitation

In our study we did not reported any case of class VI lupus nephritis. We could not examine the biopsy samples electron microscope.

Acknowledgements

The authors thank all study participants from the nephrology units and also our dedicated technicians of histopathology laboratory.

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Financial or other Competing Interests: None.