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 neutrophilic infiltration as activity predicting factors. Silent LN has been observed in class II as well as in class IV disease also. The 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.
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) neutrophilic 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 χ² 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 42.6 years (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 contrary 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 class II (figure 3), 2 cases in class III (figure 4), 17 cases in class IV (figure 5), 1 case of combine class IV and V and A single case in class V (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, class IV and combined class IV+V cases.
It was completely absent in class I, class II 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 class IV LN cases (41.1%). Cellular crescents was observed in 3 class IV LN cases (17.6%) and single class IV + V LN case (100%). Intraglomerular necrosis and karyorrhexis was found in single class IV LN cases (5.9%). Cellular crescents 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 class IV 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 infiltration 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 |         |
| 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 mesangial changes (H&E, 100X).

Fig. 3: showing eight glomeruli with mesangial 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).
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.

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 neutrophilic 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.

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