



## Vascular and Tubulointerstitial Changes in Lupus Nephritis

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### Abstract

**Background:** *Lupus nephritis affects all renal compartments namely glomeruli, tubules, interstitium and blood vessels. However the ISN/RPS classification of Lupus nephritis depends only on the glomerular changes without any assessment of tubulointerstitial and vascular changes in renal biopsies.*

**Objectives:** *1) To describe the tubulointerstitial and vascular changes in renal biopsies of six classes of lupus nephritis-ISN/RPS Classification of Lupus nephritis (2004).*

*2) To describe the immunofluorescent patterns in tubulointerstitium and blood vessels.*

### Methods:

**Study Design:** *Descriptive study*

**Study Population:** *Renal biopsies of clinically and biopsy-proven Lupus nephritis patients irrespective of age and sex which were received in the Department of Pathology, Government medical college, Kottayam during the study period.*

**Sample Size:** *37*

**Sampling Procedure:** *Continuous sampling*

**Results:** *Tubulointerstitial changes were more prevalent in Class IV cases followed by Class III. Interstitial inflammation was seen in 70% cases, Tubular atrophy in 51% cases, and interstitial fibrosis in 35% cases. Vascular changes were detected in 51% of the study population, more prevalent in Class IV and III cases. Arteriosclerosis was found in 24% cases, vascular immune deposits in 14% cases, Non-inflammatory necrotizing vasculopathy in 8% cases, and vascular thrombi in 5% cases. Tubular immune complex deposits were observed in 30% cases.*

**Conclusion:** *Since the treatment and prognosis are based on the ISN/RPS class of Lupus nephritis, we need a better assessment of each patient by seeing tubulointerstitial and vascular changes also, in addition to glomerular changes.*

**Keywords:** *Lupus nephritis, tubulointerstitial changes, vascular changes.*

### Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease affecting multiple organ systems including the skin, joints,

heart, lungs, kidneys, central nervous system, and serous membranes. The renal manifestation of SLE is called Lupus nephritis which may affect any or all renal compartments, including glomeruli, tubules,

interstitium, and blood vessels. Since the first classification of Lupus nephritis issued by WHO in 1974, it had been revised multiple times. International Society of Nephrology and Renal Pathology Society (ISN/RPS) Classification of Lupus nephritis has been widely used internationally which was revised in 2004.<sup>[1]</sup> The most recent update of the ISN/RPS Classification was published in 2018.<sup>[2]</sup> Till the latest one, all the classifications are based exclusively on an assessment of the glomerular alterations using light microscopy, immunofluorescence, and electron microscopic studies.

Tubulointerstitial lesions are seen irrespective of different classes of lupus nephritis. In general, the presence of tubulointerstitial lesions correlate well with glomerular lesions especially with Lupus nephritis classes III and IV. Tubulointerstitial lesions constitute one of the best morphologic changes that correlate with the degree of renal insufficiency. Renal vascular lesions can assume a variety of morphological forms and their presence can adversely affect the renal outcome. When vascular lesions are focally distributed, they must be systematically examined using Light microscopy, Immunofluorescence, and Electron microscopy.<sup>[1][3]</sup> Immunofluorescent staining patterns of immune deposits are often helpful in confirming a diagnosis of lupus nephritis. Immune complex deposits are detected in glomeruli, tubules, interstitium, and blood vessels which can be further evaluated for the location of electron-dense deposits by using electron microscopy.<sup>[4][5][6]</sup>

The present study aims to describe the prevalence and severity of tubulointerstitial and vascular changes in renal biopsies of different classes of Lupus nephritis patients based on light microscopy and immunofluorescence. Thus the study is based on the rationale that whether the glomerular pathology alone reflects the patient's clinical outcome.

### Objectives

1. To describe the tubulointerstitial and vascular changes in renal biopsies of six

classes of Lupus nephritis-ISN/RPS classification of Lupus nephritis(2004)

2. To describe the immunofluorescent pattern in tubulo-interstitium and blood vessels.

### Materials and Methods

**Type of Study:** Descriptive study

**Period of Study:** 16 months; November 2018 to March 2020

**Study Setting:** Department of Pathology, Govt. Medical College, Kottayam

**Sample Size:** 37

**Study Population:** Renal core biopsy specimens of 37 Lupus nephritis patients, received in the Department of Pathology, Government Medical college, Kottayam during the study period.

### Inclusion Criteria

All renal biopsies of clinically and histologically diagnosed Lupus nephritis patients irrespective of age and gender received in the Dept of Pathology, Government Medical College, Kottayam.

### Exclusion Criteria

Renal biopsies with inadequate material (number of glomeruli <5).

### Study Tools

1. Light Microscope and Immunofluorescence microscope
2. Eosin-Hematoxylin stain and special stains- Periodic Acid-Schiff, Masson's Trichrome and Jones Silver Methenamine stains.
3. For Immunofluorescence, fluorescein labeled antihuman antibodies of IgG, IgA, IgM, C3 and C1q
4. Proforma for each case

### Study Procedure

- Approval from Ethics committee and the Research Board of our institution was obtained.
- Two cores of renal tissue were obtained by percutaneous renal biopsy. Of the 2 cores, one was sent in normal saline for IF and other core was in 10% Formalin for Light microscopy.

- Light microscopy: The renal tissue obtained in 10% formalin is kept for fixation for 8 to 12 hours and then processed in a Leica CM 1510 S model histokinete and embedded in paraffin wax. Sections of 3-5 micron thickness was cut using Leica RM 2125 model rotary microtome and stained. The routine stains used were H& E , Periodic Acid Schiff, Jones Methanamine Silver and Masson's Trichrome
- Renal tissue sent in saline is frozen and is cut using ASP 300S model cryostat and stained for IgG, IgM, IgA, C3 and C1q.
- After correlating morphology and IF pattern of Immunoglobulins with the clinical and lab parameters, the final diagnosis was made.
- Based on glomerular changes, according to ISN/RPS classification 2004, cases were classified into 6 classes.
- Tubulointerstitial and vascular changes were analyzed by light microscopy and immunofluorescence.
- The following tubulointerstitial and vascular changes were described.
  - Severe: more than 50%
- Tubular atrophy: was graded as
  - Absent: 0%
  - Mild: less than or equal to 25% of the tubules
  - Moderate: 26-50%
  - Severe: more than 50%
- Tubular immune deposits: Immune deposits along tubular basement membrane.
- Vascular immune complex deposits: Immune deposits along the vessel wall in immunofluorescent study.
- Arteriosclerosis: lumen narrowing due to hyperplastic arteriosclerosis (onion skin type of intimal fibroplasia) or hyaline arteriosclerosis (medial accumulation of eosinophilic material with staining properties of fibrin).
- Non-inflammatory necrotizing vasculopathy/ Lupus vasculopathy: smudgy eosinophilic material that stains focally positive for fibrin seen to occupy the lumen and intima of arterioles, frequently extending into the media.
- Vascular thrombi: marked occlusion of lumen by thrombus.

Tubulointerstitial changes –

1. Interstitial inflammation
2. Tubular atrophy
3. Interstitial fibrosis

Vascular changes-

1. Arteriosclerosis/Arteriolosclerosis
2. Non- inflammatory necrotizing vasculopathy
3. Vascular thrombi.

Immunofluorescent microscopic study was also done using the fluorescein labeled antihuman antibodies like IgG, IgA,IgM,C3 and C1q and the patterns of immune deposits in glomeruli, tubulo-interstitium and blood vessels was observed and tabulated.

#### Definitions of Histological Parameters<sup>[1][7][8]</sup>

- Interstitial inflammation and interstitial fibrosis: were graded as
  - Absent : 0%
  - Mild: 25% or less involvement of cortical area
  - Moderate: 26-50%

#### Data Management and Analysis

The data was entered in Microsoft excel and results were expressed as mean, frequency and proportions in tables and figures.

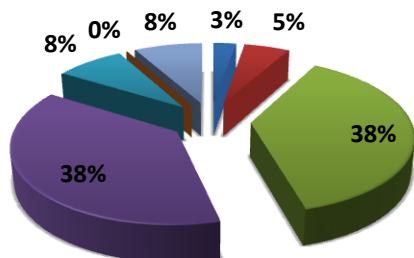
#### Results

This study was conducted on renal biopsies of 37 Lupus nephritis patients which were received in the Department of Pathology, Government Medical College, Kottayam from November 2018 to March 2020.

The mean age was  $30.9 \pm 11.3$  years, with a minimum age of 9 years and maximum age of 55 years. Maximum number of cases fell in the age group between 20-40 years (54%). 33(89%) were females and 4(11%) were males.

**ISN/RPS CLASS**

- Class I    ■ Class II    ■ Class III    ■ Class IV
- Class V    ■ Class VI    ■ Class IV+V

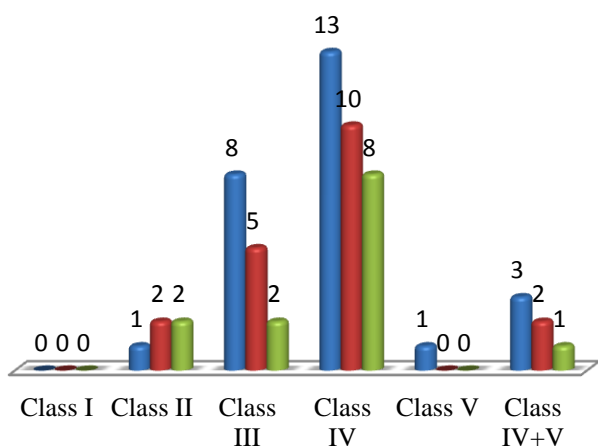


**ISN/RPS class distribution of cases**

Majority of the cases belong to Class III and IV Lupus nephritis (76%). Renal biopsy samples of Class VI lupus nephritis was not obtained during the study period. Out of 37 cases, 3 cases belonged to mixed class, Class IV+V.

**Tubulointerstitial Changes**

- Interstitial inflammation    ■ Tubular atrophy
- Interstitial fibrosis



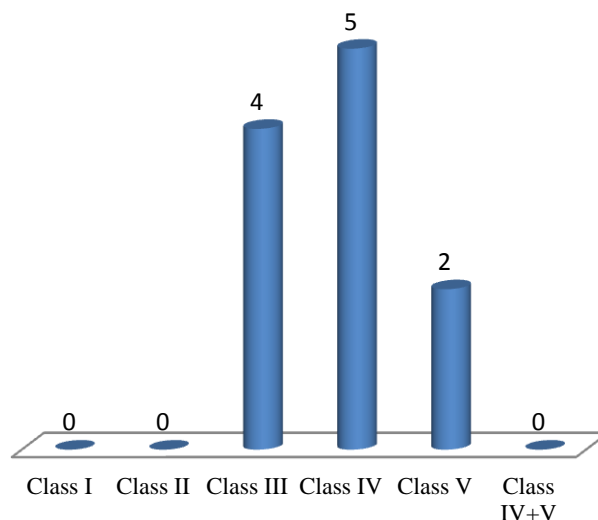
**Distribution of tubulointerstitial changes among different classes**

Most common tubulointerstitial change in the study population was interstitial inflammation (70%), followed by tubular atrophy (51%) and interstitial fibrosis (35%). All types of tubulointerstitial

changes were more common in Class IV cases followed by Class III cases.

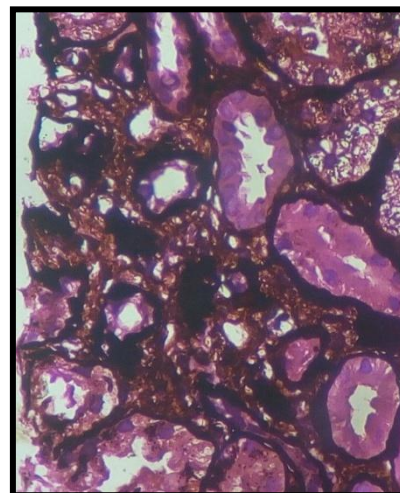
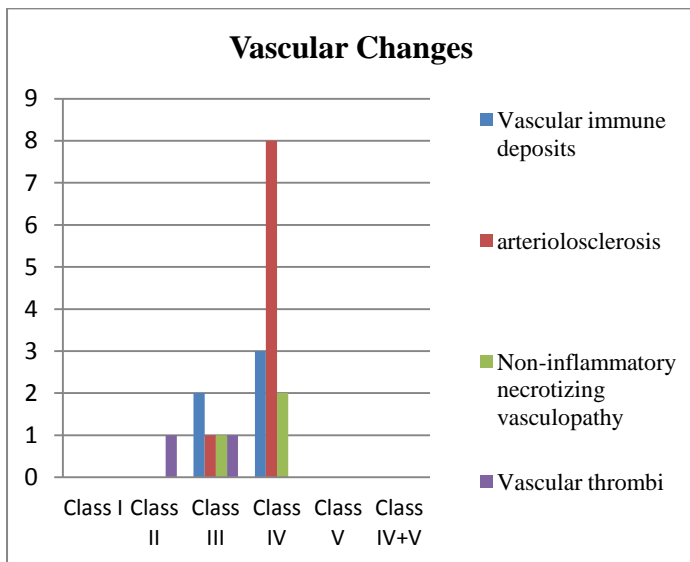
According to the study by Feng Yu et al, most severe changes observed in Class IV, moderate in Class III and mild in Class V & II.<sup>[9]</sup> Prevalence of tubulointerstitial lesions in a study conducted by Chao Yi Wu et al is 38.8%, mostly observed in Class III & IV cases (46.15%).<sup>[10]</sup>

**Tubular Immune Complex Deposits**



**Distribution of tubular immune complex deposits among different classes**

Tubular immune deposits were found in 30 % of study population. Prevalence is more among Class III and IV followed by Class V. Among the 11 cases with positive tubular immune deposits, the most common type of deposit belong to IgG followed by C1q,IgA, C3 and IgM. In the study by H.Nossent et al, 48% of cases showed tubular immune deposits.<sup>[11]</sup>



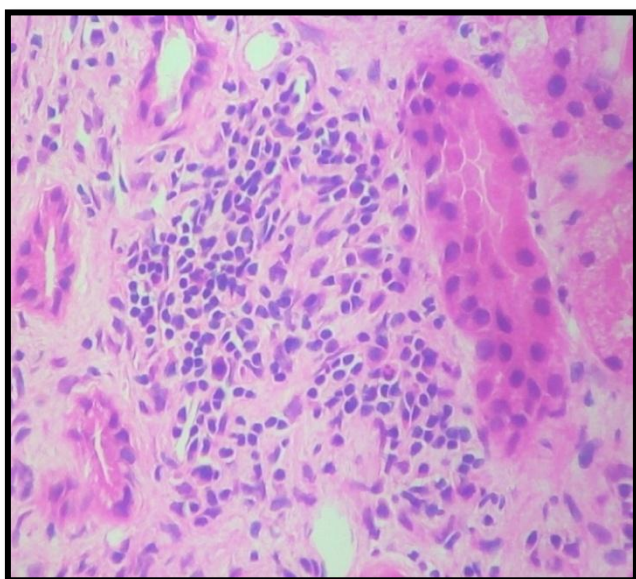
**Tubular atrophy Jones Methenamine Silver stain- (Class IV) 400 X**

**Distribution of vascular changes among different classes**

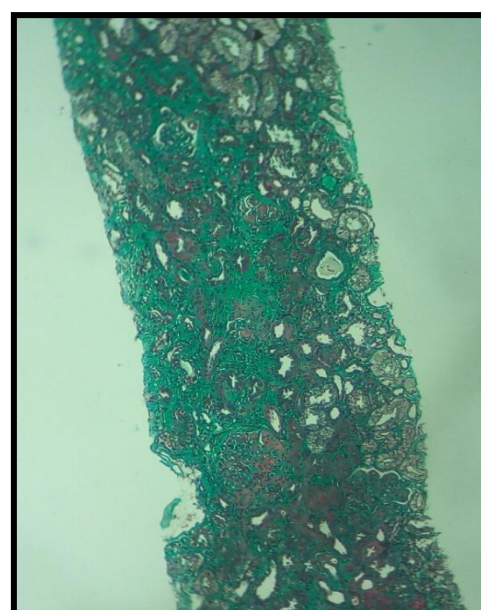
Prevalence of vascular lesions in the present study was 51% which is comparable with that of JM Meji Vilet et al<sup>[12]</sup> which was 53.4%.

Most common vascular change encountered in the study population is arteriolosclerosis (24%) followed by vascular immune deposits(14%), non-inflammatory necrotizing vasculopathy(8%) and vascular thrombi(5%). Most common type immune complex deposit is IgG followed by C1q, IgM among those with positive vascular immune deposits.

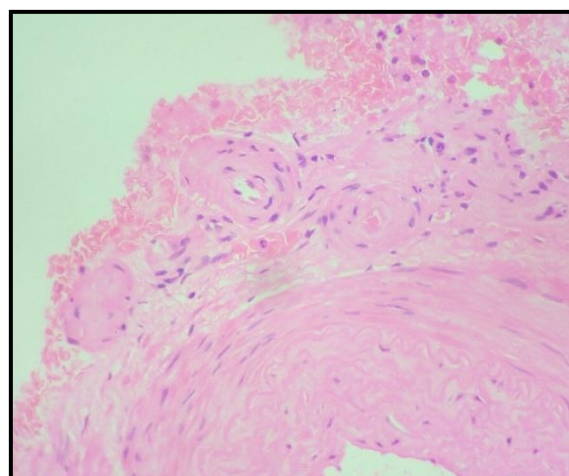
**Photomicrographs**



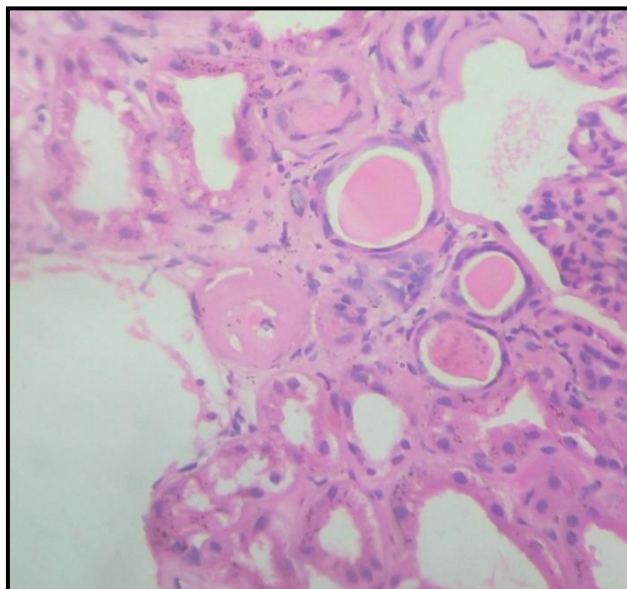
**Interstitial inflammation composed of lymphocytes & plasma cells-(Class IV) 400X**



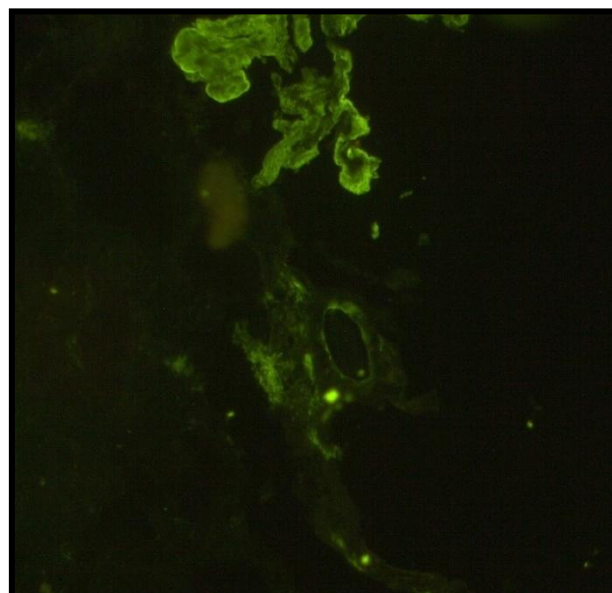
**Interstitial fibrosis –Masson Trichrome stain- (Class IV) 100X**



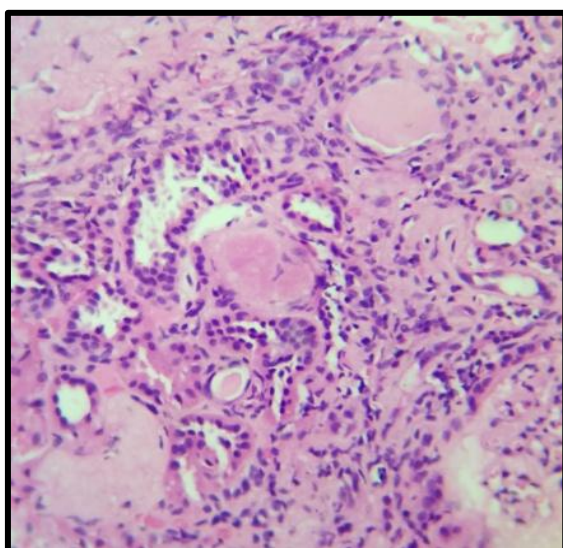
**Arteriolosclerosis with occlusion of some blood vessels- (Class IV+V) 400X**



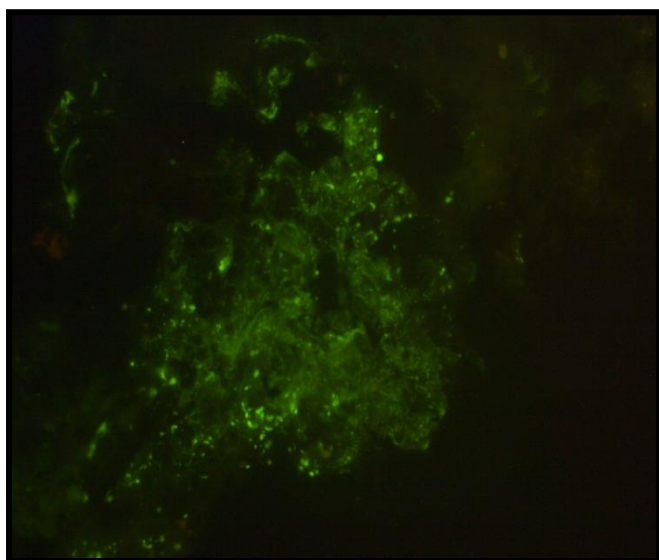
Vascular hyaline thrombus-(Class III) 400X



Blood vessels: IgM deposits- (Class IV)



Lupus vasculopathy-(Class IV+V) 400X



Tubules: C1q deposits – (Class IV)

### Conclusion

- There were significant tubulointerstitial and vascular changes in different classes of Lupus nephritis, which was more frequent in Class IV and III cases.
- Interstitial inflammation was found in 70% of the cases, followed by Tubular atrophy in 51% and interstitial fibrosis in 35% cases.
- Prevalence of vascular lesions in the study population was 51%, namely arteriolosclerosis (24%), vascular immune deposits (14%), non-inflammatory necrotizing vasculopathy (8%) and vascular thrombi (5%).
- With immunofluorescence, 30% of the cases showed tubular immune deposits and 14% cases showed vascular immune deposits, most commonly IgG followed by C1q positivity in tubules as well as vessels.

Since the treatment and prognosis of Lupus nephritis patients are according to ISN/RPS class solely based on glomerular changes, we need a better assessment of each lupus nephritis patient by giving significance to tubulointerstitial and vascular changes also.

### Acknowledgement

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