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Original Research Paper

Pathology

A STUDY OF LUPUS NEPHRITIS IN RENAL BIOPSIES IN AN INDIAN TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Lupus Nephritis poses greatest risk of morbidity and mortality with most demanding therapeutic challenge. Renal biopsy plays a very important in management of patients with SLE. The study aims to evaluate the incidence of LN, demography, clinical and laboratory, histological findings and proportion of patients in each class of Lupus Nephritis based on the International Society of Nephrology/Renal Pathology Society classification. **Methods:** This is a retrospective study, carried over a period of two years. All cases of SLE were classified based on 2012 SLICC classification and 1997 ACR classification. Renal biopsies showing more than 5 glomeruli were reviewed and classified. **Result:** Out of 65 (22.73%) cases of LN, there was female preponderance (94%). Mean age at presentation was 30.21 years (+11.81). Proteinurea was the most common clinical finding. Mixed class of Lupus nephritis was reported in majority cases (25%). **Conclusion:** The ISN/RPR-2003 classification provided a significant advantage in handling of renal biopsies of SLE patients, with a striking inter and intra-observer reproducibility.

KEYWORDS : ISN/RPR (2012), ACR (1997), SLICC (2012).

1. INTRODUCTION

The renal manifestation of systemic lupus erythematosus (SLE) called "Lupus Nephritis", is highly pleomorphic with respect to its clinical and morphological expressions.The renal complications poses the greatest risk of morbidity and mortality, and also present the most demanding therapeutic challenge.The onset of renal involvement is most common within the first year, clinically affecting up to 50% of patients with SLE[1]. Renal biopsy plays an important role in the management of patients with SLE, especially early in the disease, before overt extrarenal manifestations of SLE are evident. This scenario applies most frequently to patients with mesangial proliferative or membranous patterns who lack serological markers of SLE, and may present many months or even years before the American College of Rheumatology (ACR) criteria for SLE have been met[2]. The International Society of Nephrology/Renal Pathology Society (ISN/RPS) proposed a classification for LN that is periodically updated over a period of time.

2. AIM AND OBJECTIVES

The study aims to evaluate the incidence of LN, demographic, clinical and laboratory, histological findings and proportion of patients in each class of lupus nephritis in a tertiary care hospital of Western Maharashtra.

3. MATERIAL AND METHODS

Retrospective study, carried out in the Department of Pathology, KEM Hospital, Mumbai, over a period of two years, including 65 cases of LN. The data regarding age, gender, etiology, clinicopathological correlation, light microscopic features and relevant laboratory investigations were recorded from histopathology reports. All the renal biopsies showing more than 5 glomeruli were reviewed and classified according to the 2003 INS/RPS Classification of LN. All the cases of SLE were classified based on 2012 SLICC (Systemic Lupus International Collaboration Clinics) classification and 1997 ACR (American College of Rheumatology Clinics) classification criteria for SLE. Continuous variable like age was presented as mean (+standard deviation), and other variables like percentage of LN on renal biopsy, sex, class of LN, clinical presentation, immunlogical findings, associated co-morbid conditions, were presented as numbers (frequency).

4. RESULTS

Out of total 286 kidney biopsies, 65 (22.73%) cases diagnosed as LN on light microscopy were included. Most of the LN cases were in the age range of 21 to 30 years (34%) followed by 11 to 20 years (23%). Mean age was 30.21 years (+ 11.81). There was female preponderance (94%) with Male to Female ratio 1:15.25.

According to the SLICC classification criteria the most common clinical feature at presentation was proteinuria in 49 cases out of 65 (75.38%), followed by oral ulcers in 24 out of 65 cases (36.92%). Antinuclear antibody values were available in 35 cases, out of which 33 cases (94.28%) showed positive titers and 2 cases (5.72%) were ANA negative. Anti-dsDNA values were available in 31 cases, out of which 16 cases (51.62%) showed positive Anti-dsDNA titers and 15 cases (48.38%) were Anti-dsDNA negative. Serum complement levels C3 were available in 32 cases and C4 in 30 cases. The normal range of Serum C3 is 90-180mg/dl. It was low in 31 out of 32 cases (97%) and normal in 1 cases (3%). The normal range of Serum C4 is 10-40 mg/dl. It was low in 22 out of 30 cases (73%) and normal in 8 cases (27%).

Table 1: Distribution Of Cases Bases On The 2012 Slicc Classification Criteria For Sle

SR NO.	CRITERIAS	Number Of Cases (n=65)	Percentage
CLINICAL CRITERIA			
1	Acute Cutaneous Lupusss	18	27.69
2	Chronic Cutaneous Lupus	02	3.07
3	Oral ulcers	24	36.92
4	Non-scarring alopecia	15	23.07
5	Synovitis involving >2 joints	21	32.30
6	Serositis	12	18.46
7	Renal manifestation (Proteinuria)	49	75.38
8	Neurological manifestation	01	1.53
9	Hemolytic anemia	03	4.61
10	Leucopenia/Lymphopenia	09	13.84
11	Thrombocytopenia	05	7.69
IMMUNOLOGICAL CRITERIA			
1	ANA (n=35)		
	ANA Positive	33	94.28

	ANA Negative	02	05.72
2	Anti-dsDNA (n=31)		
	Anti-dsDNA Positive	16	51.62
	Anti-dsDNA Negative	15	48.38
3	Anti-Sm	00	00
4	Anti-Phospholipid Antibody	00	00
5	Low Complement		
	Low C3 (n=32)	31	97
	Low C4 (n=30)	22	73
6	Direct Coombs Test	12	18.46
Classify a patient as having SLE if a) The patient satisfies four of the criteria, including at least one clinical criterion and one immunological criterion OR b) The patient has biopsy-proven nephritis compatible with SLE and with ANA or anti-dsDNA antibodies			

According to the ACR classification criteria for SLE the most common feature at presentation was positive ANA in 33 out of 35 cases (94.28%) followed by proteinuria in 49 out of 65 cases (75.38%).

Table 2: Distribution Of Cases Bases On The 1997 Acr Classification Criteria For Sle

SR NO.	CRITERIAS	Number Of Cases (n=65)	Percentage
1	Malar Rash	18	27.69
2	Discoid Rash	01	1.53
3	Photosensitivity	05	7.69
4	Oral ulcers	24	36.92
5	Nonerosive Arthritis	21	32.30
6	Pleuritis or Pericarditis	12	18.46
7	Renal disorder (Proteinuria)	49	75.38
8	Neurologic Disorder	01	1.53
9	Hematologic disorder • Hemolytic anemia Or • Leucopenia/ • Lymphopenia Or • Thrombocytopenia	 03 09 05	 4.61 13.84 7.69
10	Immunologic disorder Anti-DNA antibody to native DNA positive (n=31)	16	51.62
11	Positive antinuclear antibody (n=35)	33	94.28
Classify a patient as having SLE if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.			

In this study, Mixed class of Lupus Nephritis was seen in majority of cases i.e 16 out of 65 cases (25%), followed by Class III LN in 13 out of 65 cases (20%) and Class IV 13 out of 65 cases (20%)

Table 3: Distribution Of Cases According To Isn/rps 2003 Classification Of Ln

CLASS	Number Of Cases (n=65)	Percentage (%)
CLASS I	00	0
CLASS II	09	14
CLASS III	13	20
CLASS III (C)	10	-
CLASS III (A/C)	03	-
CLASS IV	13	20
CLASS IV-S (C)	02	-
CLASS IV-S (A/C)	09	-
CLASS IV-G (A/C)	02	-
CLASS V	12	18
CLASS VI	02	03
MIXED CLASSES	16	25
CLASS II & III (C)	01	-
CLASS II & V	01	-
CLASS III (A/C) & V	01	-
CLASS IV-S (C) & V	01	-
CLASS IV-S (A/C) & V	06	-
CLASS IV-G (A/C) & V	03	-
CLASS V & VI	01	-
CLASS V progressing to VI	02	-
TOTAL	65	100

In this study Mixed connective tissue disease was the most common associated condition in 7 out of 65 cases (10.76%), followed by Secondary Sjogren's syndrome in 5 out of 65 cases (7.69%) and autoimmune hemolytic anemia in 2 out of 65 cases (3.07%)

Table 4: Distribution Of Cases Based On Associated Autoimmune Disease (n=65)

Associated Autoimmune Diseases	Number Of Cases	Percentage (%)
Mixed connective tissue disease	07	10.76
Secondary Sjogren's syndrome	05	7.69
Autoimmune hemolytic anemia	02	3.07
Scleroderma	01	1.53
Rheumatoid arthritis	01	1.53
Dermatomyositis	01	1.53
Anti-phospholipid syndrome	01	1.53
Immune thrombocytopenic purpura	01	1.53

According to the 2012 SLICC Classification Criteria of SLE, Renal manifestation is defined as urine protein/creatinine (or 24 hours urine protein) representing 500 mg of protein in 24 hours or red blood cell casts. In this study 24 hours urine protein was quantified in 35 cases out of 65, and not quantified in 14 cases out of 65. There was no data available in 16 cases out of 65. The highest mean protein excretion was found in Class V LN i.e 2.88 gm/24 hours (0.85-6.73 gm/24 hours), followed by mixed class of LN i.e 2.77gm/24 hours (0.6-7.45 gm/24 hours).

5. DISCUSSION

This study assessed the demographic, clinical, basic laboratory characteristics and distribution of cases in various histological class of LN, in a Western Maharashtra tertiary care hospital. A comparison with studies available in literature has been done.

In this study, the percentage of LN in kidney biopsies on light microscopy was 22.73 %, which was similar to done by Dr. Neethu Kishor et al [25%] (2016)[3] and Kosaraju,et al [20.83%] (2010)[4] in southern part of India. The mean age of patients in this study was 30.21years (+11.81), and the age range of 10-60 years. This result was similar to studies of Randa I. Farha et al [29.95 +12.16] (2019)[5] , Ana Karla Guedes de Melo et al [26.78 +10.95] (2009)[6] years. In study done by Keya Basu et al (2020)[7] on pediatric lupus nephritis, where the mean age was 15.12 years (+3.49) and 12.5 years (+1.73) for proliferative and non-proliferative lupus nephritis. In this study, of 65 cases 4 were male (6%) and 61 were female (94%).

There was female preponderance (94%) with Male to Female ratio 1:15.25. This finding was similar to study done by Randa I. Farha et al (2019)[5] where out of 79 cases, 11(13.9%) were male and 68 (86.1%) were female, with male to female ratio of 1:6. In this study, Mixed class of LN was seen in majority of cases i.e. 16 out of 65 cases (25%), followed by Class III LN in 13 out of 65 cases (20%) and Class IV 13 out of 65 cases (20%). The most common mixed pattern of LN was Class IV+V, which was noted in 10 out of 65 cases (15.38%). This study showed dissimilar findings compared to below mentioned studies in literature.

Table 5: Comparitive Study Of Case Distribution.

SR. NO	Class of LN	Studies					
		Current Study		Randa I. Farha et al (2019)5		Dr.Suchitha Satish et al(2017)8	
		NO.	%	NO.	%	NO.	%
1	Class I	0	0	02	2.50	0	0
2	Class II	09	14	05	6.30	4	7.1
3	Class III	13	20	10	12.70	13	23.2
4	Class IV	13	20	37	46.80	31	55.4
5	Class V	12	18	15	19.00	07	12.5
6	Class VI	02	03	01	1.30	01	1.8
7	Mixed Classes	16	25	07	8.90	-	-

In a study by Randa I. Farha et al, Class IV LN accounted for 46% cases, followed by 19% cases of Class V LN. Mixed pattern of LN was seen in 8.90% cases. In a study by Dr. Suchitha Satish et al[8], Class IV LN accounted for 55% cases, followed by 23.2% cases of Class II LN. In this study the most common clinical feature at presentation was proteinuria in 49 cases out of 65 (75.38%), followed by oral ulcers in 24 out of 65 cases (36.92%). This study showed dissimilar findings compared to studies done by Dr. Manjuri Sharma et al (2019)[9], where pedal edema (92%) was most common clinical feature at presentation, followed by anemia (72.1%), and Dr. Shobha Vineeta et al (2014)[10], where pedal edema (68.7%) was the most common clinical feature at presentation, followed by proteinuria (62.5%). In this study ANA values were available in 35 cases out of 65, of which 33 (94.28%) cases showed positive titers and 2 (5.72%) cases were ANA negative. Anti-dsDNA values were available in 31 cases out of 65, out of which 16 (51.62%) cases showed positive Anti-dsDNA titers and 15 (48.38%) cases were Anti-dsDNA negative. Serum complement levels C3 was available in 32 cases and C4 in 30 cases. C3 was low in 31 out of 32 cases (97%) and normal in 1 case (3%). C4 was low in 22 out of 30 cases (73%) and normal in 8 cases 8 cases (27%). This study showed 2 ANA negative SLE cases. One was a 17-year-old female, a known case of Class IV LN, dialysis dependent presented with facial puffiness, breathlessness and pallor. On examination, bipedal edema, ascites, oliguria was noted. She was clinically diagnosed as case of LN with acute kidney injury, and renal biopsy was done to look for change of class of LN. Her serological findings were 24 hrs Protein- 340mg/24 hrs, serum creatinine 3 mg/dl, C3 was low, C4 was normal, Serum ANA and Anti-dsDNA were negative. On light microscopy she was reported as Class IV-S (A/C) LN with 50-60% IFTA. The second case was a 25-year-old female, a known case of Class V LN, with Azathioprine induced bone marrow suppression. Her renal biopsy was done in view of persistent proteinuria more than 2gm & microscopic hematuria to decide for plan of immunosuppression. Her serological findings were 24 hrs Protein- 2.4 gm /24 hrs, serum creatinine 1.9 mg/dl, C3 & C4 were normal, Serum ANA and Anti-dsDNA were negative. On light microscopy she was reported as Class V LN with 20-25% IFTA. In study done by Dr. Clement Wilfred Devadas et al (2010)[11] serum ANA titers were positive in 82% cases. In study done by Eman M Farid et al (2013)[12] serum ANA titers were positive in 100% cases. ANA positivity is one of the criteria in the American College of Rheumatology's criteria and SLICC criteria for classification of SLE. The description of ANA-negative lupus was first raised by Stephen R. Koller et al. in 1976[13]. He described five patients who were ANA-negative but had clinical features consistent with SLE. In study done by L.S .Cross et al (2004)[14] stated that true ANA negative lupus is an extremely rare event. F N Ozdemir et al.(2005)[15] described a 28-year-old female who was admitted with acute renal failure following her fourth delivery. The serum immunological markers were negative and renal biopsy showed histopathological changes consistent with SLE as the etiology of her nephrotic syndrome. In a study by Hyoun-Ah Kim et al.(2009)[16] patients with clinical features of SLE with negative test for ANA appear to represent 1-5% of the SLE population. ANA-negative could be because of technical inaccuracy, short follow up period, or the renal damage in few patients may not have been mediated by auto-antibodies that do not react with ANA[16]. In this study 18.46% cases (12/65) had a positive Coomb's test. Only 25% of those with positive Direct Coomb's test (i.e. 3 out of 12 cases) developed autoimmune hemolytic anemia. In study done by N. Abou Assalie et al (2015)[17] showed 20% of patients with SLE had a positive Coombs test. Only 38% of those with the positive Direct Coombs test developed an autoimmune hemolytic anemia. The study concluded that those cases of SLE with positive Direct Coombs test who develop hemolytic anemia are more likely to have renal or CNS-SLE, and Direct Coomb's positivity that leads to hemolytic anemia have particularly poor prognosis. In this study Mixed connective tissue disease was the most common associated condition in 7 out of 65 cases (10.76%), followed by Secondary Sjogren's syndrome in 5 out of 65 cases (7.69%) and autoimmune hemolytic anemia in 2 out of 65 cases (3.07%).

The present study showed dissimilar findings compared to the study done by Dr. Sharon Chambers et al (2007)[18] where Sjogren's syndrome was most common associated autoimmune disease in SLE patients (11.2%), followed by Antiphospholipid syndrome (5.7%), concluding that physicians should be aware that patients with SLE might present with a variety of other autoimmune diseases which could have an impact on treatment and mortality. The physicians should not necessarily attribute all of patients signs and symptoms to SLE only. In study done by J E McDonagh et al (2000)[19], Sjogren's syndrome was most common associated autoimmune disease in SLE patients (13%), followed by Rheumatoid arthritis. In this study 24 hours urine protein was quantified in 35 cases out of 65. The highest mean protein excretion was found in Class V LN i.e. 2.88 gm/24 hours (0.85-6.73 gm/24 hours), followed by mixed class of LN i.e. 2.77gm/24 hours (0.6-7.45 gm/24 hours). This study showed dissimilar findings compared to the studies Bijay Bartaula et al (2019)[20], the mean 24 hours urine protein was highest in Class VI LN (4.70gm/24 hrs), followed by Class V LN (3.99gm/24 hrs) and Dr. Suchitha Satish et al[8], the mean 24 hours protein was highest in Class VI LN (2.95 gm/24 hrs), followed by Class IV LN (2.33gm/24 hrs).

Limitations of this study are as follow: The sample size of study was small, making correlation of clinical and laboratory data with histopathological type difficult. The study was based on a single center design with very limited associated data available. To understand the risk factor and pathogenesis of LN better in our geographical area, a common disease registry and more comprehensive and uniform interpretation of results among different patients is recommended.

6. CONCLUSION

Lupus nephritis continues to be the major source of morbidity and mortality for SLE patients. In this study 65 renal biopsies were analyzed with respect to demographic findings, SLICC and ACR classification criteria for SLE and various laboratory investigations. The study categorized cases of LN based on ISN/RPS- 2003 classification, following which mixed class/overlapping pattern of LN was the most frequent type seen in the study. The ISN/RPS classification provided significant advances in the handling of renal biopsies of SLE patients. The most striking advantage of the classification is higher inter observer and intra-observer reproducibility resulting from a uniform reporting system used around the world. The week point of the classification is that it does not include nonglomerular compartment such as tubulointerstitium which may also be related to the prognosis. Thus, the classification needs to be improved and clarified with additional studies in order to interpret the potential pathogenetic relevance of the classes.

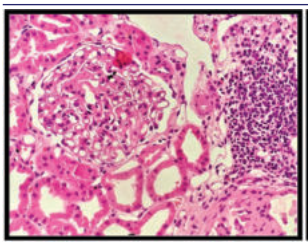

Image 1: (h & E 400x) Class V Ln – Showing Glomeruli With Thick Capillary Walls And Lymphoplasmycytic Infiltrate In Background.

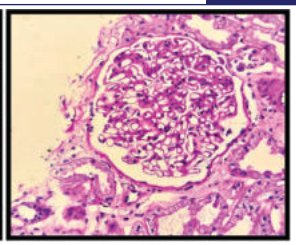

Image 2: (pas 400x) Class V Ln- Showing Glomeruli With Thick Capillary Walls

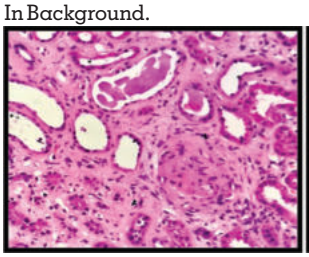

Image 3: (h & E 400x) Class Vi Ln- Showing A Globally Sclerosed Glomerulus With Interstitial Fibrosis & Tubular Atrophy In Background.

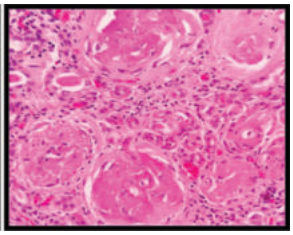

Image 4: (h & E 400x) Class Vi Ln-showing A Globally Sclerosed Glomeruli.

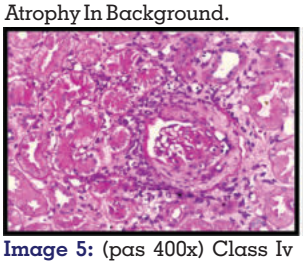

Image 5: (pas 400x) Class Iv Ln- Gluomerulus Showing Fibrocellular Crescent.

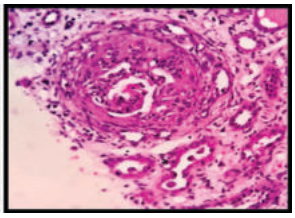

Image 6: (h & E 400x) Class Iv Ln- Glomerulus Showing Fibrocellular Crescent With Ifta In Background.

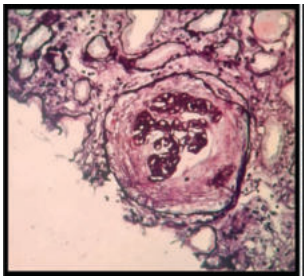

Image 7: (sm 400x) Class Iv Ln- Glomerulus Showing Fibrocellular Crescent.

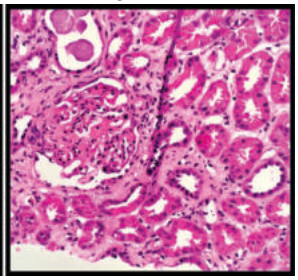

Image 8: (h & E 400x) Class Iv Ln- Glomerulus Showing Wire Loop Lesion.

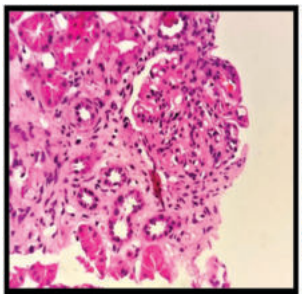

Image 9: (h & E 400x) Class Iv Ln-glomeruli Showing Increases Mesangialcellularity, With Increased Endocapillary Cellularity & Segmental Sclerosis.

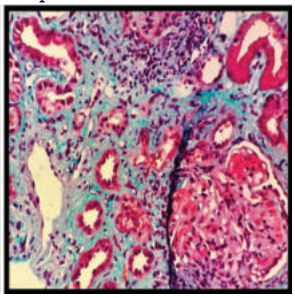

Image 10: (masson Trichrome 400x) Class Iv Ln Showing Interstial Fibrosis And Tubular Atrophy.

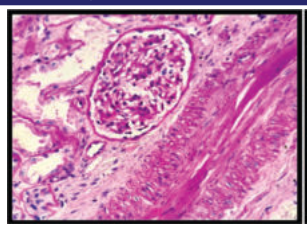

Image 11: (pas 400x) Class Iii Ln- Glumerulus Showing Segmental Sclerotic Lesion.

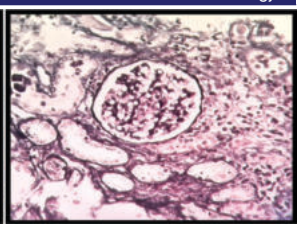

Image 12: (sm 400x) Class Iii Ln- Glumerulus Showing Segmental Sclerotic Lesion.

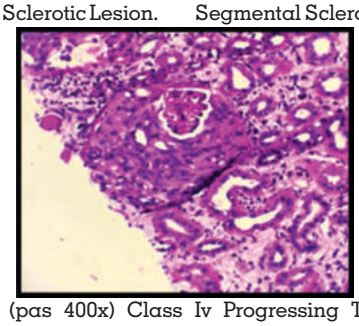

Image 13: (pas 400x) Class Iv Progressing To Class Vi- Glomerulus Showing Cellular Crescent.

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