ejpmr, 2021,8(8), 562-569.

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

A STUDY ON CORRELATION BETWEEN LEVEL OF PROTEINURIA AND DIFFERENT CLASSES OF LUPUS NEPHRITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS ADMITTED IN A TERTIARY CARE HOSPITAL IN EASTERN INDIA

[1]Dr. Boudhayan Bhattacharjee, [2]*Dr. Arindam Naskar, [3]Dr. Sudeshna Mallik, DR. Anuradha De (Pati) and [5]Prof. Bibhuti Saha

[1]M.B.B.S, MD, RMO Cum Clinical Tutor, Dept. of General Medicine, Medical College and Hospital, Kolkata 700073, India.

[2]M.B.B.S., MD, MRCP (UK), Assistant Professor, Dept of Tropical Medicine, School of Tropical Medicine, Kolkata 700073, India.

[3]M.B.B.S., MD, Associate Professor, Dept of Tropical Medicine, School of Tropical Medicine, Kolkata 700073, India.

[4]M.B.B.S., MD, Associate Professor, Dept of Pathology, Calcutta National Medical College and Hospital, Kolkata 700073 India.

[5]M.B.B.S., DTM & H, MD, Professor & Head, Dept of Tropical Medicine, School of Tropical Medicine, Kolkata 700073, India.

***Corresponding Author: Dr. Arindam Nasakar**

M.B.B.S., MD, MRCP (UK), Assistant Professor, Dept of Tropical Medicine, School of Tropical Medicine, Kolkata 700073, India.

Article Received on 08/06/2021 **Article Revised on 29/06/2021** **Article Accepted on 19/07/2021**

ABSTRACT

Background: Systemic lupus erythematosus is an auto-immune condition where renal involvement is an important predictive marker of mortality which mainly occurs in first decade of the disease. **Objective:** Aim of this study was to diagnose lupus nephritis early and to correlate the extent of renal involvement with different levels of proteinuria. **Method:** In this single center, cross-sectional study in the year 2015-2017 total 32(thirty-two) cases were taken as cases of SLE, diagnosed according to Current Systemic Lupus International Collaborating Clinic Criteria For Classification Of Systemic Lupus Erythematosus and all underwent 24 hours urinary protein estimation followed by renal biopsy. **Results:** A weak positive correlation was found between level of proteinuria and classes of lupus nephritis (correlation co- efficient 0.259; 95% CI -0.09866 to 0.5573; significance level 0.0152). Best cut-off value for proteinuria for doing renal biopsy to detect high grade lupus nephritis (class III or higher) using the ROC curve was found to be 234 mg (sensitivity 88.00% and specificity 71.43%). Our study found that 65% of the patients had class IV lupus nephritis [class IV B > class IV A] followed by class II (22%), class V (9%) and Class III (3%). **Conclusion**: So, a SLE patient presenting with more than 234 mg of protein in 24 hours urinary sample should undergo renal biopsy for early detection of high grade lupus nephritis which will need higher immunosuppressive therapy.

KEYWORDS: Proteinuria, Renal Biopsy, Lupus Nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is an auto-immune condition where the body cells and organs undergo damages by tissue binding autoantibodies and immune complexes. Ninety percent of patients are women of child bearing age; but people of all ages, gender and ethnic groups are susceptible with a 9:1 female preponderance,[1] Several auto-antibodies have been documented in SLE. Amongst them anti-double stranded DNA (anti-dsDNA) and anti-Smith (anti- Sm) are most specific for SLE. Anti-nuclear antibody (ANA) is positive in >98% patients during the course of illness. Auto- antibodies has been shown to have predilection to specific organ affection, like Anti-dsDNA in lupus nephritis. Systemic Lupus International Collaborating Clinic Criteria For Classification Of Systemic Lupus Erythematosus proposed criteria for diagnosing SLE,[2] (TABLE 1). Presence of four criteria (one each in each category) qualifies the patient to be classified as having SLE with 93% specificity and 92% sensitivity. Only renal biopsy suggesting lupus nephritis without meeting any other criteria can be regarded as SLE.

Pattern and degree of injury obtained from renal biopsy are indicators for best therapy. Lupus nephritis is a serious complication of SLE occurring in 50% patients diagnosed as SLE,[3] Renal involvement is an important predictive marker of mortality in SLE,[4] Mortality from

renal involvement mainly occurs in first decade of the disease. Clinical presentation of lupus nephritis varies from asymptomatic to haematuria or proteinuria or rapidly progressive glomerulonephritis with loss of renal function. It may also present as nephrotic syndrome. Renal involvement is commonly seen within 36 months of disease. Several forms of renal involvements have been documented in SLE. These are immune complex mediated glomerulonephritis (most common), tubulo-interstitial disease and vascular disease. Lupus nephritis is defined as clinical and laboratory criteria that meet ACR criteria i.e. persistent proteinuria >500 mg/day or greater than 3+ by dipstick and/ or presence of cellular casts including RBC, haemoglobin, granular, tubular, or mixed. The optimal criterion for diagnosis is renal histopathology findings of an immune complex mediated glomerulonephritis as interpreted by an experienced pathologist employing accepted criteria and rating of activity and chronicity should be noted.[5,6] According to international Society of Nephrology/ Renal Pathology Society Classification lupus nephritis can described in six classes.[7]

These are

A) Who Class I: Minimal Mesangial Lupus Nephritis: Normal glomeruli by light microscopy but immune deposits by immunofluorescence.

B) Who Class II: Mesangial Proliferative Nephritis: Mesangial hyper-cellularity or matrix expansion with immune deposits on light microscopy. Few isolated sub-epithelial or sub-endothelial deposits on immunofluorescence.

C) Focal Lupus Nephritis: Active or inactive, focal, segmental, or global endocapillary or extra capillary glomerulonephritis involving <50% of all glomeruli, typically with focal sub-endothelial immune deposits with or without mesangial alterations.

D) Class Iv: Diffuse Lupus Nephritis: Active or inactive, focal, segmental, or global endocapillary or extra capillary glomerulonephritis involving >50% of all glomeruli, typically with diffuse sub-endothelial immune deposits with or without mesangial alterations; a. Diffuse segmental (IV-S) - >50% of involved glomeruli has segmental lesions, b. Diffuse global (IV-G)- >50% of involved glomeruli has global lesions; Segmental lesions are lesions that involves less than half of glomerular tuft.

E) Class V: Membranous Lupus Nephritis: Global or segmental sub-epithelial deposits or their morphologic sequalae by light microscopy and Immunofluorescence. Class V lupus nephritis can occur in combination with class III or IV, in those cases both will be diagnosed. Class V lupus nephritis may have advanced sclerosis.

F) Class Vi: Advanced Sclerotic Lupus Nephritis: >90% of all glomeruli are globally sclerosed without any residual activity.

A renal biopsy may depict a single class of nephritis or a combination two or three classes. Class III and IV is called 'proliferative' due presence endocapillary proliferation within the glomeruli. Thick endothelial lesions form classical "wire loop" lesions that is seen in class IV lupus nephritis. Class V nephritis is commonly associated with nephrotic range proteinuria. The WHO class IV has the highest prevalence followed by Class III, II and V in descending order. Presence of tubulo-reticular inclusion bodies within endothelial cells strongly suggestive of lupus nephritis. The characteristic features of lupus nephritis are sometimes referred to as the "full-house" pattern, because IgG, IgM, IgA, C3, and C1q are all found in the deposits. Based on clinical features, the degree of urine albumin, abnormal urinary sediments, presence or absence of hypertension, and renal failure, it is not possible to predict the histopathological subtypes.[8,9] Because nephritis is asymptomatic in most lupus patients, urine analysis and early renal biopsy play a crucial role in diagnosing lupus nephritis.

Accurate measurement of 24 hours proteinuria is important for predicting glomerular damage. Patients having chronic kidney disease have shown that the magnitude of proteinuria is a strong predictor of glomerular filtration rate decline[10]. Normal protein in urine is excreted is less than 15 mg/day. 24 hours urine collection methodical error may result in under or over estimation of protein level in urine. Serum creatinine is an unreliable marker for decline in GFR. Hematuria can be absent in patients with severe class IV nephritis, and proteinuria may be modest in patients with class V nephritis.[11] These are Prevalence of clinical manifestations in patients with lupus nephritis,[12] Proteinuria- 100%, Nephrotic range proteinuria/nephrotic syndrome- 50%, Microscopic haematuria-80%, Macroscopic haematuria- <5%, Urinary RBC casts-30%, Other urinary cellular casts- 30%, Renal insufficiency- 60%, Rapid decline in kidney function-15%, Hypertension- 30%, Tubular abnormalities- 70%.

These are indications for renal biopsy in patients with systemic lupus erythematosus[13]

1. Increasing serum creatinine without compelling alternative causes (such as, sepsis, hypovolemia, or medication) [Level C recommendation] *
2. Confirmed proteinuria of 1.0 gm per 24 hours (either 24-hour urine specimens or spot protein/creatinine ratios are acceptable) [Level C recommendation]*
3. Combinations of the following, assuming the findings are confirmed in at least 2 tests done within a short period of time and in the absence of alternative causes: [Level C recommendation]*

- Proteinuria 0.5 gm per 24 hours plus haematuria, defined as 5 RBCs per HPF
- Proteinuria 0.5 gm per 24 hours plus cellular casts

*Level A evidence represents data derived from multiple RCTs or a meta-analysis, Level B evidence represents

data from a single RCT or nonrandomized study, and Level C evidence represents data from consensus, expert opinion, or case series.

3. AIMS AND OBJECTIVES OF STUDY

A) To diagnose lupus nephritis early
B) To correlate the extent of renal involvement with different levels of proteinuria

4. METHODS AND MATERIALS

The study protocol was submitted to the clinical Research Ethics Committee of Calcutta School of Tropical Medicine, Kolkata for ethics review and approval. The study started after ethical clearance.

The patients who were admitted in the inpatients department (IPD) of Carmichael Hospital for Tropical Diseases (CHTD), Calcutta School of Tropical Medicine, Kolkata from July 2015 to June 2017 and were diagnosed as a case of systemic lupus erythematosus (according to Current Systemic Lupus International Collaborating Clinic Criteria For Classification Of Systemic Lupus Erythematosus) and after fulfilling inclusion and exclusion criteria, 32 (thirty-two) subjects were taken as cases.

Inclusion Criteria

1. Patients those are fulfilling criteria of defined study population
2. Patients aged more than 12yrs,
3. Patient gives consent to participate in the study.

Exclusion Criteria

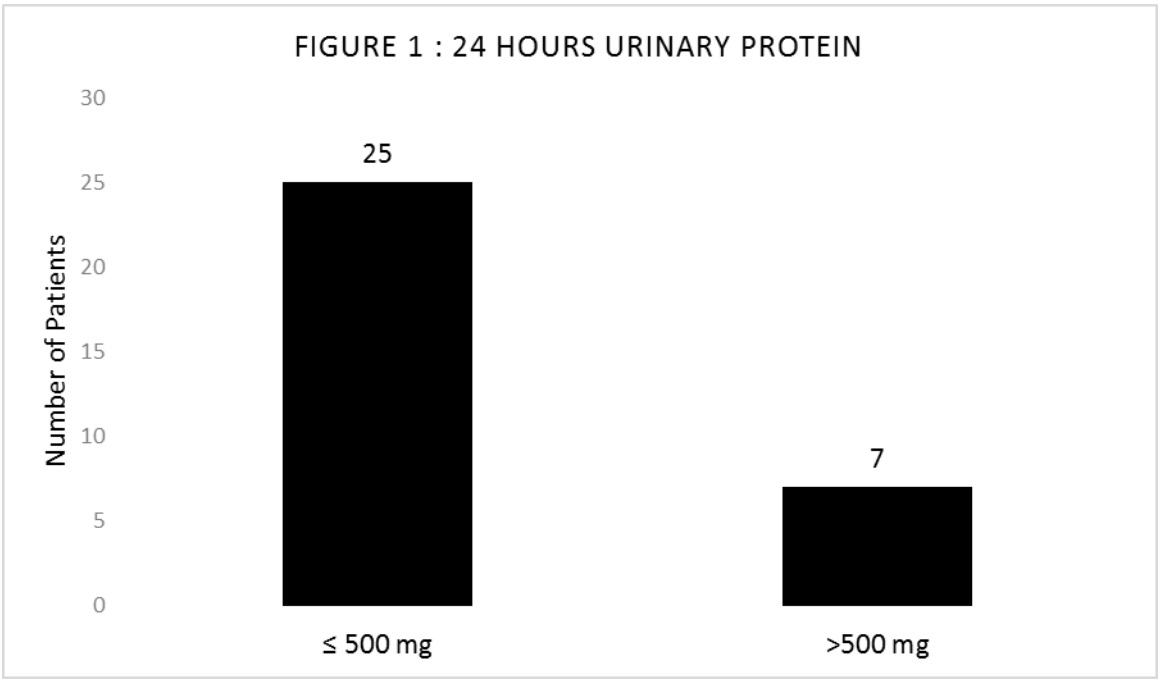
1. Patient having other diseases that causes proteinuria (like urinary tract infection, diabetes mellitus etc.).
2. Patient aged less than 12yrs.
3. Pregnant women, patients with contraindication to renal biopsy (like bleeding diathesis, malformations of kidney, urinary tract infection etc.).
4. Patients with significant co-morbidities e.g. Diabetes, malignant diseases, HIV, cardio-respiratory illness, Asthma, liver disease, chronic kidney disease, diagnosed patient of Systemic Lupus Erythematosus on therapy, patient of Overlap syndrome associated with SLE.

All the patients were subjected to detailed assessment including focused interview and history elicitation with an emphasis on medication history, relevant clinical examination and investigations. For all patients routinely 24 hours urinary protein level was measured by automated urine protein analyser at the time of diagnosis of SLE. Patients were classified in two groups high-level proteinuria i.e. >500 mg/day and low-level proteinuria i.e. ≤500/day). Renal biopsy was done in patients with indications of renal biopsy (even patients with low proteinuria with other indications of renal biopsy were taken, like increased number of RBCs in urine, raised creatinine level). During renal biopsy two cores were taken- one for histopathological examination (formalin fixed) and the other for immunofluorescence study (Immunofluorescence fluid). Core for histopathology prepared for paraffin embedded section which was stained by Haematoxylin and eosin stain. These sections are then analysed under light microscope. Core for Immunofluorescence study made into sections by cryostat. These sections were immediately assessed by antibody panel (IgG, IgM, IgA, IgE, IgD, complements). Morphological study and result of Immunofluorescence study did classification of Lupus Nephritis according to international Society of Nephrology/Renal Pathology Society Classification lupus nephritis. Now classes of Lupus nephritis were correlated according to proteinuria level.

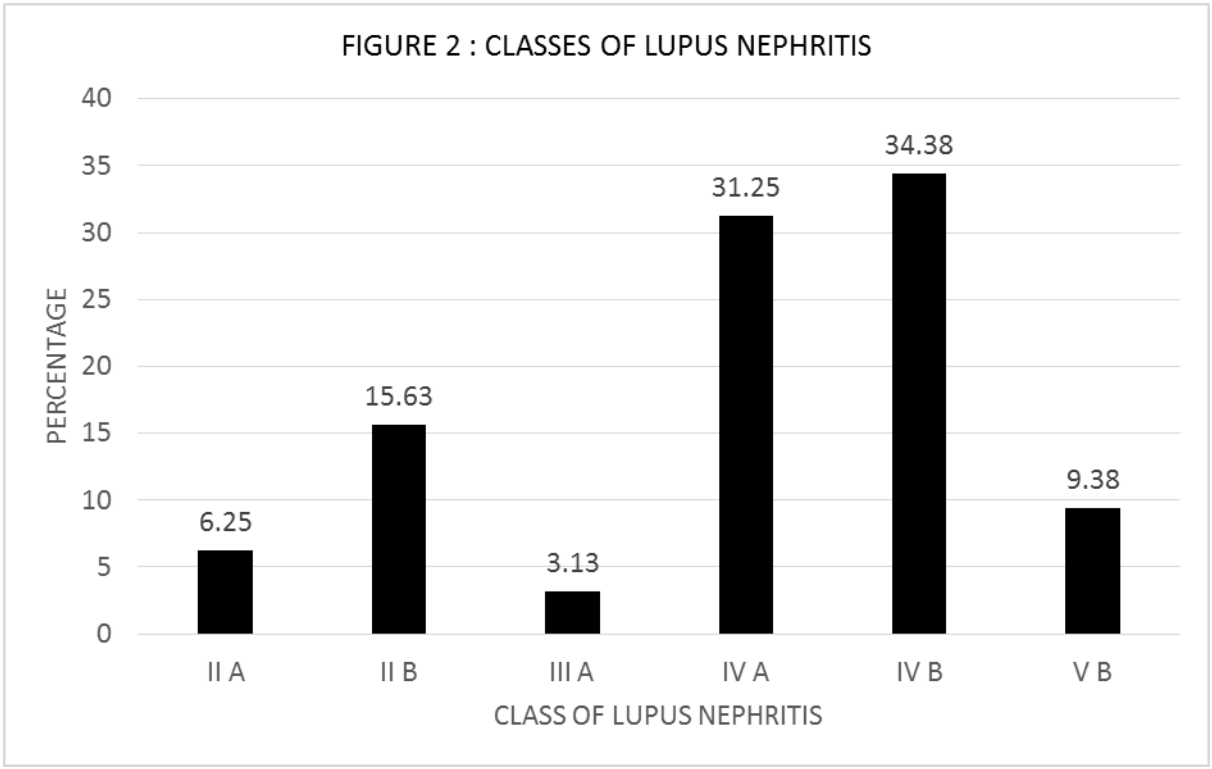
Data was tabulated and analysed according to appropriate statistical methods. Informed consent process was undertaken in accordance with the ICMR Guidelines. Risks, if any and benefit of study participation was explained to the patient/legal representative during the administration of informed consent process. Written consents were obtained from patients and /or legal representative. In case of a patient/legal representative who cannot provide informed consent in writing, a left thumb print to indicate consent in the presence of at least one witness was taken as acceptable.

5. RESULTS AND ANALYSIS

A) 24 Hours Urinary Protein: Mean of 24 hours urinary protein was 425.19 ± 342.34. Out of 32 patients 25 (78%) patients had proteinuria of less than 500 mg in 24 hours. No patient had nephrotic range proteinuria (>3g/day). (Figure 1).

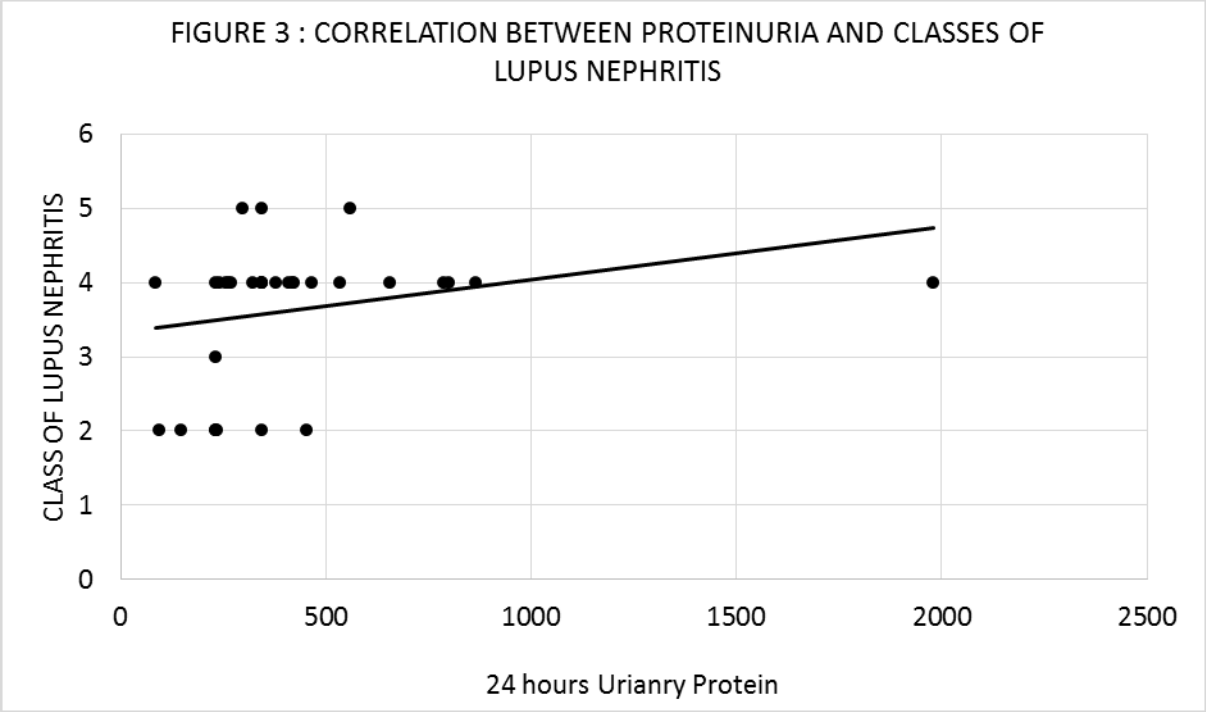


B) Classes of Lupus Nephritis: Majority of patients (65%) had class IV lupus nephritis with class IV B marginally greater than class IV A. (Figure 2).



C) Correlation Between Level Of Proteinuria And Classes Of Lupus Nephritis

There is weak positive correlation between level of proteinuria and classes of lupus nephritis. Correlational statistics showed correlation co- efficient 0.259 (95% CI -0.09866 to 0.5573); significance level 0.0152. (Figure 3).



The best cut-off value for 24-hour urinary proteinuria for doing renal biopsy to detect high grade lupus nephritis (class III, IV and V) using the ROC curve was 234 mg (sensitivity 88.00% and specificity 71.43%) and the area under the ROC curve was 0.771 ± 0.0110 (95% CI: 0.589–0.900, $P = 0.0133$) (Figure 4)

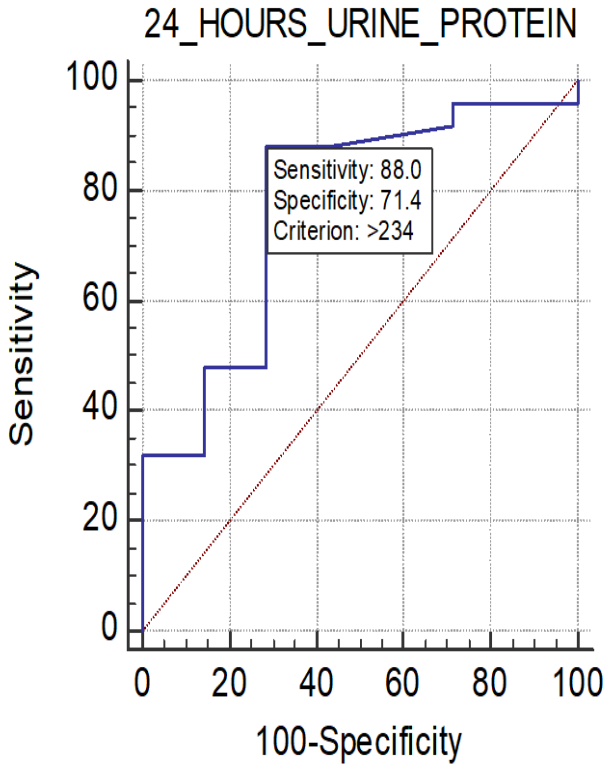
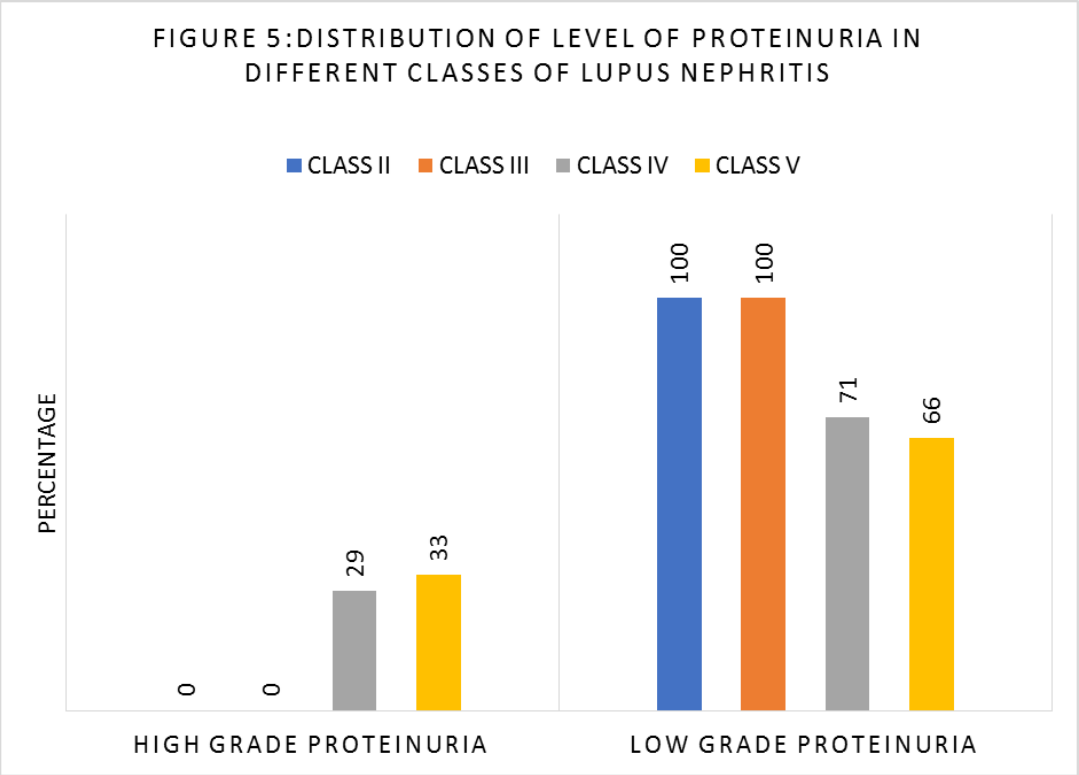


Figure 4: Roc Curve Of Proteinuria.

D) Distribution of Level of Proteinuria in Different Classes of Lupus Nephritis (Figure 5)



High grade proteinuria= Urinary protein more than 500 mg in 24 hours
Low grade proteinuria= Urinary protein less than or equals to 500 mg in 24 hours

1. High Grade Proteinuria: Urinary protein level more than 500 mg in 24 hours were present in only 29% and 33% of patients with class IV and class V nephritis respectively. And no high-grade proteinuria was present in class II and III lupus nephritis patients.
2. Low Grade Proteinuria: Urinary protein less than or equals to 500 mg in 24 hours were present in 100%, 100%, 71%, 66% of patients with class II, III, IV and V lupus nephritis respectively.

6. DISCUSSION

We found mean of 24 hours urinary protein was 425.19 ± 342.34. Out of 32 patients 25(78%) patients had proteinuria of less than 500 mg in 24 hours. No patient had nephrotic range proteinuria (>3g/day). On renal biopsy majority of patients (65%) had class IV lupus nephritis [class IV B marginally greater than class IV A] followed by class II (22%), class V (9%) and Class III (3%). Jacobsen S et al.[14] showed in a study with 94 SLE patients that renal biopsy is very important to diagnose proliferative lupus nephritis (diffuse proliferative or membrano-proliferative) because proteinuria or haematuria are poor predictor of renal pathology. A. van Tellingen et al.[15] suggested indication and importance of doing renal biopsy in patients with low level of proteinuria (<0.5g/24hrs) with or without microscopic hematuria. Mahajan et al.[16] recommends not to perform a renal biopsy in SLE patients having a normal renal function, no haematuria and <0.5 g/24 hours of proteinuria. Our study revealed that there is a weak positive correlation between level of proteinuria and classes of lupus nephritis. Correlational statistics showed correlation co- efficient 0.259 (95% CI -0.09866 to 0.5573); significance level 0.0152. The best cut-off value for 24-hour urinary proteinuria for doing renal biopsy to detect high grade lupus nephritis (class III, IV and V) using the ROC curve was 234 mg (sensitivity 88.00% and specificity 71.43%) and the area under the ROC curve was 0.771 ± 0.0110 (95% CI: 0.589–0.900, $P = 0.0133$). Class III, IV and Class V lupus nephritis has been regarded as high-grade nephritis and class II is low grade nephritis. So, a SLE patient presenting with more than 234 mg of protein in 24 hours urinary sample should undergo renal biopsy for early detection of high grade lupus nephritis which will need higher immunosuppressive therapy. Lisa Christopher-Stine et al.[17] studied 21 SLE patients with 24-h urine protein < 1000 mg who underwent kidney biopsies and found that significant renal involvement (Class III, IV, or V LN) in SLE patients with < 1000 mg proteinuria with or without haematuria suggesting that biopsy be strongly considered in SLE patient population.

Wen YK et al.[18] retrospectively analysed renal biopsy findings in 131 SLE patients who had undergone renal biopsy within 3 months from the diagnosis of SLE. And they found that patients presenting with sub-nephrotic proteinuria, half of patients had proliferative Lupus Nephritis (class III, IV, mixed class V + III) and the other half of the patients had non-proliferative lupus nephropathy (class II, pure class V). In patients presenting with nephrotic range proteinuria, proliferative

LN (class III, IV, mixed class V + III) and non-proliferative lupus nephropathy (class II, pure class V) accounted for 55% and 36% of patients, respectively, whereas 9% had non-lupus nephropathy. Our study found that urinary protein level more than 500 mg in 24 hours (high grade proteinuria) were present in only 29% and 33% of patients with class IV and class V nephritis respectively. And no high-grade proteinuria was present in class II and III lupus nephritis patients. Urinary protein less than or equals to 500 mg in 24 hours (low grade proteinuria) were present in 100%, 100%, 71%, 66% of patients with class II, III, IV and V lupus nephritis respectively. So, high grade lupus nephritis (class III or greater) patients have high grade proteinuria in only approx. 30% of patients. On the contrary low-grade lupus nephritis (class II or lesser) have all patients (100%) with low grade proteinuria.

7. CONCLUSION

Aim of this study was to diagnose lupus nephritis earlier and to find out correlation between proteinuria and classes of lupus nephritis. Our study found that 65% of the patients had class IV lupus nephritis [class IV B > class IV A] followed by class II (22%), class V (9%) and Class III (3%). There was weak positive correlation between level of proteinuria and classes of lupus nephritis. High grade proteinuria (> 500 mg/24 hours) was present in only 29% and 33% of patients with class IV and class V nephritis respectively. Best cut-off value for proteinuria for doing renal biopsy to detect high grade lupus nephritis using the ROC curve was 234 mg (sensitivity 88.00% and specificity 71.43%). So, a SLE patient presenting with more than 234 mg of protein in 24 hours urinary sample should undergo renal biopsy for early detection of high grade lupus nephritis which will need higher immunosuppressive therapy.

8. REFERENCES

1. Cohen-Solal JF, Jeganathan V, Hill L, et al: Hormonal regulation of B-cell function and systemic lupus erythematosus, *Lupus,* 2008; 17(6): 528– 532.
2. Petri et al: *Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus,* Arthritis Rheum, 2012; 64(8): 2677.
3. L. M. Ortega, D. R. Schultz, O. Lenz, V. Pardo, and G. N. Contreras, "Lupus nephritis: pathologic features, epidemiology and a guide to therapeutic decisions," Lupus, 2010; 19(5): 557–574.
4. Danila MI, Pons-Estel GJ, Zhang J, et al: Renal damage is the most important predictor of mortality within the damage index: data from LUMINA LXIV, a multiethnic US cohort, *Rheumatology (Oxford),* 2009; 48: 542–545.
5. Tan EM,Cohen AS,Fries JF,Masi AT,McShane DJ,Rothfield NF,Schaller JG,Talal N,Winchester RJ. *The revised criteria for the classification of systemic lupus erythematosus*. Arthritis Rheum, *1982;* 25(11): 1271–1277.
6. Dooley MA,Aranow C,Ginzler EM.*Review of ACR renal criteria in systemic lupus erythematosus.* Lupus, 2004; 13(11): 857–860.
7. JJ Weening et al: *The classification of glomerulonephritis in systemic lupus erythematosus revisited.* Kidney Int., 2004 Feb; 65(2): 521-30.
8. Shobha V,Prakash R,Arvind P,Tarey SD, *Histopathology of lupus nephritis: A single-center, cross-sectional study from Karnataka, India*, Internet Journal of Clinical Immunology and Rheumatology, 2014; 2(S1): OA3.
9. Jacobsen S, Starklint H, Petersen J, et al: Prognostic value of renal biopsy and clinical variables in patients with lupus nephritis and normal serum creatinine, *Scand J Rheumatol*, 1999; 28: 288–299.
10. Keane WF: Proteinuria: its clinical importance and role in progressive renal disease, *Am J Kidney Dis.*, 2005; 35(4 Suppl 1): S97-S105.
11. DALL'ERA M,WOFSY D,chapter 80,clinical features of systemic lupus erythematosus, ,heading Renal biopsy,Kelley's textbook of rheumatology, editors Gary S. Firestein,Ralph C. Budd,Sherine E. Gabriel, Iain B. McInnes, JamesR.O'dell,ninth edition, 2013; II: 1291.
12. Salem Almaani, Alexa Meara, and Brad H. Rovin. Update on Lupus Nephritis: Clin J Am Soc Nephrol, November 2016. doi: 10.2215/CJN.05780616.
13. Bevra h. Hahn, maureen a. Mcmahon, alan wilkinson, w. Dean wallace, david i. Daikh, john d. Fitzgerald, george a. Karpouzas, joan t. Merrill, daniel j. Wallace, jinoos yazdany, rosalind ramsey-goldman, karandeep singh, mazdak khalighi, soo-in choi, maneesh gogia, suzanne kafaja, mohammad kamgar, christine lau, william j. Martin, sefali parikh, justin peng, anjay rastogi, weiling chen, and jennifer m. Grossman: American College of Rheumatology Guidelines for Screening, Treatment, and Management of Lupus Nephritis: Arthritis Care and Research June 2012; 64(6): 797–808.
14. Jacobsen S,Starklint H,Petersen J et al:Prognostic value of renal biopsy and clinical variables in patients with lupus nephritis and normal serum creatinine, Scand J Rheumatol, 1999; 28: 288–299.
15. A. van Tellingen, A.E. Voskuyl, M.G. Vervloet, M. Bijl, R.G.L. de Sévaux, S.P. Berger, R.H.W.M. Derksen, J.H.M. Berden,Dutch guidelines for diagnosis and therapy of proliferative lupus Nephritis,on behalf of the Dutch Working Party on Systemic Lupus Erythematosus,The Netherlands journal of medicine, May 2012; 70(4).
16. Mahajan SK, Ordonez NG, Feitelson PJ, Lim VS, Spargo BH, Katz AI. Lupus nephropathy without clinical renal involvement. Medicine, 1977; 56: 493-500.
17. Lisa Christopher-Stine, Mark Siedner, Janice Lin, Mark Haas, Hemal Parekh, Michelle Petri and Derek M Fine. Renal biopsy in lupus patients with low levels of proteinuria. The Journal of Rheumatology, February 2007; 34(2): 332-335.

18. Wen YK, *Renal biopsy findings in new-onset systemic lupus erythematosus with clinical renal disease*,Division of Nephrology, Department of Internal Medicine, Changhua Christian Medical Center, 135,Nanhsiao Street, Changhua 500,Taiwan,Int Urol Nephrol.2011 Sep;43(3):801 6.doi:10.1007/s11255-011-9911-3.Epub, 2011 Feb 20.