Correlation of Glomerular C4d Staining with Disease Activity in Lupus Nephritis- A prospective study

Bheemavathi.A*, K.Durga*, Swarnalata Gowrishankar**

* Upgraded Department of Pathology, Osmania Medical College
** Department of Pathology, Apollo Health city campus, Hyderabad

Abstract **-**The purpose of this study is to evaluate whether glomerular C4d deposition can be a useful marker of disease activity in lupus nephritis. This is a 2 years study conducted from May 2010 to April 2012. Renal biopsies from 40 patients diagnosed as lupus nephritis are taken . Lupus nephritis incidence is more common in females (90%) and the mean patient age is 26±12.9 years. The most common clinical presentation of lupus nephritis is nephrotic syndrome (67.5%). Lupus nephritis cases are classified according to ISN/RPS 2003. Class I-2 cases, class II-4 cases, class III-3 cases, class IV-12, class V-11, class VI-1, class V+IV-5 and class V+III-2 cases. Full house pattern of immunoflourescence is seen in 50% of the cases. C4d - complement factor 4 breakdown product is detected by immunohistochemistry. It showed positivity in 70% of the cases. Analysis of C4d deposition with histological activity index is done, which is not significant statistically (p value 0.4).

Index Terms - Activity index, glomerular C4d, lupus nephritis, marker of disease activity.

I. INTRODUCTION

Systemic lupus erythematosus (SLE) is the prototype of a multisystem disease of autoimmune origin, characterized by a vast array of autoantibodies, particularly antinuclear antibodies (ANAs). Acute or insidious in its onset, it is a chronic, remitting and relapsing, often febrile illness characterized principally by injury to the skin, joints, kidney, and serosal membranes. Ninety percent of cases are woman usually of child bearing age.[1]

Renal involvement is a frequent and a potentially serious complication of systemic lupus erythematosus that may influence morbidity and mortality. Renal biopsy findings are valuable in facilitating assessment and management of patients with lupus nephritis (LN) by confirming the diagnosis, evaluating the disease activity and suggesting prognosis and appropriate therapy.

C4d, a cleavage product of the activated complement component C4, has attained considerable significance in the last few years for its role in helping elucidate the pathophysiology of renal allograft rejection. C4d is the most clinically used marker for humoral rejection.[2]

Compliment split products are more sensitive indicators of disease activity than conventionally measured C3 and C4. [3-11] Serum C4d levels correlate with degree of disease activity.[5] However, there are fewer studies on correlation between tissue C4d deposition and disease activity in lupus nephritis.

The purpose of this study is to evaluate whether glomerular C4d deposition can be a useful marker of disease activity in lupus nephritis.

II. RESEARCH ELABORATION

2.1 Aim of the study:

-To evaluate whether glomerular C4d deposition can be a useful marker of disease activity in lupus nephritis.
-To study the patterns of C4d deposition in native kidneys of patients with lupus nephritis.

2.2 Materials and methods:

Material:

The present study c4d staining in lupus nephritis was conducted from May 2010 to April 2012 in the Upgraded department of pathology, Osmania Medical College, Hyderabad and department of pathology, Apollo hospital, Hyderabad. Renal biopsies sent from Department of Nephrology, Osmania Medical College, Hyderabad were taken into this study.

Methods:

Clinical parameters are retrieved from medical records. The indications for renal biopsy are evaluation of nephritic syndrome, nephritic syndrome and rapidly progressing renal failure. 40 renal biopsy cores were sent in Bouin's fixative for light microscopy (LM) and tissue was wrapped in a saline soaked gauze and transported in ice for immunoflourescence (IF).

For LM, tissues were processed routinely by automated tissue processor and stained by Hematoxylin and Eosin (H&E) , Periodic acid and Schiff (PAS) and silver stains.

IF was done with following FITC labeled antibodies (from Dako Inc. Denmark)
Anti IgG (FITC-F-0202)
Anti IgM (FITC-F-0203)
Anti IgA (FITC-F-0204)
Anti C3c (FITC-F-0201)
Anti C1q (FITC-F-0254)
Anti Kappa light (FITC-F-0198)
Anti Lamda light chain (FITC-F-0199)

Immunohistochemistry was done with Polyclonal Rabbit anti-Human C4d antibody (Biogenesis) (Catalog Number: 0300-0230),

III RESULTS

3.1 Age distribution

Age group ranged from 0-60 years. Lupus nephritis incidence was more common in 21 to 30 age group which constituted 35% followed by 11-20 age group which constituted 27.5%.

3.2 Gender distribution

Among 40 cases, 36 were females which constituted 90%.

3.3 Clinical presentation

Patient's clinical manifestations were broadly grouped into 3 categories based upon their presentation as nephrotic syndrome, nephritic syndrome and rapidly progressing renal failure (RPRF). Nephrotic syndrome patients (62.5%) had proteinuria greater than 3.5gm/day in adults, >50mg/kg/day in children, hypercholesterolemia and edema. Nephritic syndrome patients (17.5%) presented with hematuria, hypertension and oliguria. RPRF patients(20%) had serum creatinine levels greater than 5mg/day and patients were dialysis dependent.18 patients (45%) had elevated serum creatinine levels (> 1.5 mg/dl) and 20 patients (50%) had 24 hours protienuria greater than 500mg/day.

3.4 Classification[12]

Table 1: Abbreviated International Society of Nephrology/ Renal Pathology Society (ISN/RPS) classification of lupus nephritis (2003)

Class I Minimal mesangial lupus nephritis
Class II Mesangial proliferative lupus nephritis
Class III Focal lupus nephritis
Class IV Diffuse segmental (IV-S) or global (IV-G) lupus nephritis
Class V Membranous lupus nephritis
Class VI Advanced sclerosing lupus nephritis

.
Class V may occur in combination with class III or IV in which case both will be diagnosed.

In the present study ,Class I-2 cases, class II-4 cases, class III-3 cases, class IV-12, class V-11, class VI-1, class V+IV-5 and class V+III-2 cases were diagnosed.

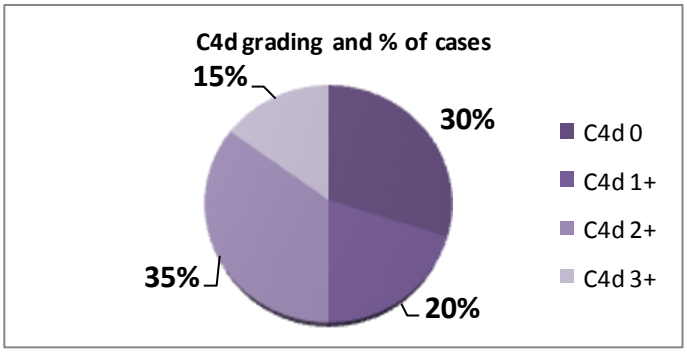
3.5 Immunoflourescence (IF)

IF study of IgG, IgM, IgA, C3, C1q, Kappa and Lamda chains immune deposition was done for all the cases.
Full house pattern of immune deposition was seen in 55% of the cases, followed by deposition of IgG, IgM, C3, C1q and IgG, C3, C1q in 17.5% of the cases. Immune deposits were localized in peripheral, mesangial or diffuse. Pattern of immune deposits also varied, mostly fine granular pattern.

3.6 Immunohistochemistry (IHC)

C4d deposition was graded as 0, 1+, 2+ and 3+. C4d immune deposition was not observed in 12 cases (30%), 1+ in 8 cases (20%),2+ in14 cases (35%) and 3+ in 6 cases (15%). 28 cases (70%) showed deposition of C4d in the glomerular structures. No peritubular capillary c4d deposition was observed. C4d deposition was graded as 0, 1+, 2+ and 3+ based on intensity. Pattern of deposition was coarsely granular, linear along the entire capillary circumference

Figure 1 : C4d grading and % of cases



3.7 Disease activity in Lupus Nephritis

In addition to the pathologic classification, activity and chronicity indices are scored pathologically and predict the renal prognosis-that is, the progression of renal disease.

The activity index reflects the state of active inflammation observed at biopsy, which may be reversible with medical therapy. The chronicity index reflects the amount of fibrosis and scarring, which are unlikely to respond to therapy.

The activity and chronicity indices are evaluated at a single point in time and renal lesions may transform from one class to another either spontaneously or as a result of treatment.

Activity index was done for 22 cases belonging to class III (3), IV (12), V+IV (5) and V+III (2). Activity index was graded as mild (0-7), moderate (8-14) and severe (15-21) according to ISN/RPS.

Table 2: Active and chronic glomerular lesions (ISN / RPS 2003)[12]

Activity Index	Chronicity Index
Endocapillary hypercellularity with or without leukocyte infiltration; luminal reduction Karyorrhexis Fibrinoid necrosis Rupture of glomerular basement membrane Crescents, Cellular or fibrocellular crescents Subendothelial deposits on light microscopy (wireloops) Intraluminal immune aggregates(hyaline thrombi)	Glomerular sclerosis; (segmental, global) Fibrous adhesion Fibrous crescents

Each variable is scored 0 - 3 points. Total activity is 21 and chronicity is 9.

Score	**Activity (total 21)**	**Chronicity (total 9)**
Mild	1 – 7	1 – 3
Moderate	8 – 14	4 – 6
Severe	15 – 21	7 – 9

Statistical analysis was done by Pearson chi-square test, p value 0.479.

Table 3: Analysis of activity index (AI) with C4d grading

AI	**C4d 0**	**1+**	**2+**	**3+**	**Total**
Mild	3	2	3	2	10
Moderate	1	4	2	-	7
Severe	-	-	-	-	0
No activity	2	1	3	-	6

Figure 2: Class III-Focal lupus nephritis- Endocapillary hypercellularity & wireloops (PAS 40x)

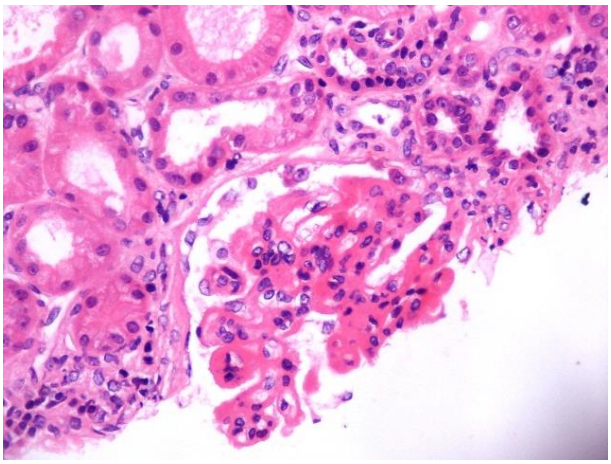


Figure 3: Hyaline thrombi – Class IV (PAS 40x)

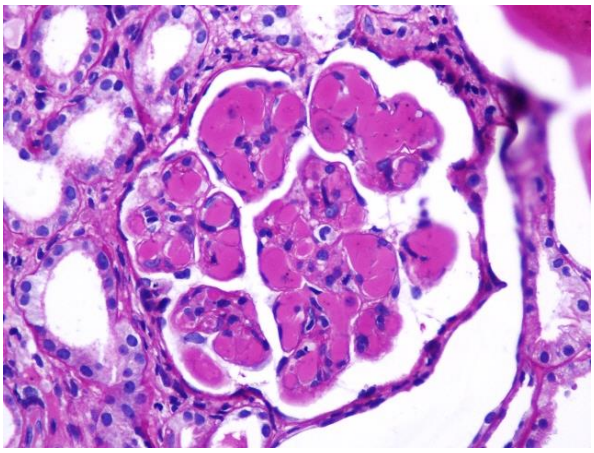


Figure 4: Full house pattern of immunofluorescence

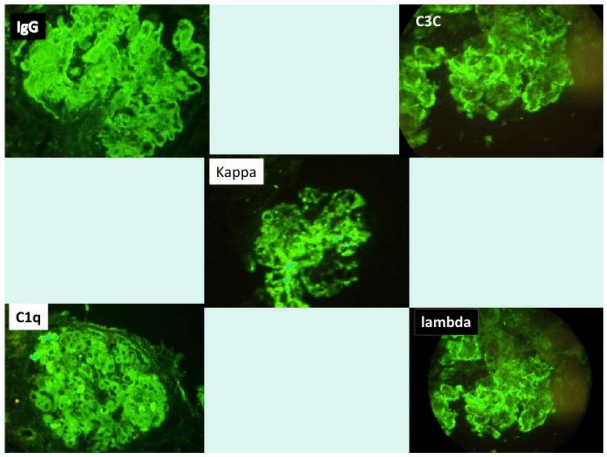
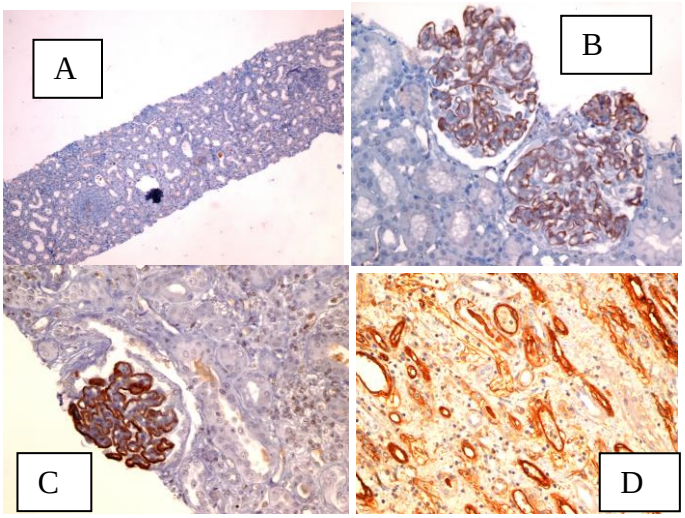


Figure 5: Different intensities of glomerular C4d staining. A. No glomerular C4d staining. B. 2+ glomerular C4d staining C. Intense 3+ glomerular C4d staining D. Control-Renal allograft biopsy showing peritubular capillary deposition



IV DISCUSSION

Complement component 4 is a protein involved in the complement system. It is cleaved into proteins 4a and 4b.

- C4a is an anaphylatoxin.
- C4b forms part of C3-convertase, in conjunction with 2a
- C4d is the final proteolytic remnant of deposited C4b on endothelium, remains covalently attached to endothelium and easily detectable by antibody staining.

C4d is the most clinically used marker for humoral rejection. It is a degradation product of the activated complement factor C4b. C4d is typically initiated by binding of antibodies to specific target molecules. Following activation and degradation of the C4 molecule, thio-ester groups are exposed, which allow transient, covalent binding of the degradation product C4d to endothelial cell surfaces and extracellular matrix components of vascular basement membranes near the sites of C4 activation[13]. C4d is also found in intra cytoplasmic vacuoles of endothelial cells. Covalent binding renders C4d a stable molecule that can easily be detected by immunohistochemistry. In addition, C4d may also be generated by the antibody independent mannan binding lectin pathway.

Kim and Jeong et al (2003) studied glomerular C4d deposition in 21 cases diagnosed as lupus nephritis. Immunoflourescence for C4d showed diffusely granular staining along glomerular capillary loops, in all cases examined (1+ in 8 cases; 2+ in 7

cases; 3+ in 6 cases). The intensity of C4d correlated with those of capillary IgG, IgA, C4, C1q, and fibrinogen. However, C4d staining intensity did not correlate with the lupus nephritis activity index[14].

Sumanth R. Daram et. al (2006) studied patterns of C4d staining in lupus nephritis. All 16 patients (100%) showed deposition of C4d in the glomerular structures, and one also had focal deposition of C4d in the peritubular capillaries. class II (4 cases), class III (3 cases), class IV (8 cases), and class V (1 case) [15].

Sahin OZ et al observed a relationship between glomerular C4d staining and activity of lupus nephritis. C4d staining may be a useful marker to predict the prognosis of lupus nephritis. Among total 24 cases, fourteen (58%) patients were C4d+ and 10 (42%) patients C4d-. Urinary protein excretion was more elevated in C4d+ group ($p = 0.0001$). The renal biopsy showed that activity index score >12 was a higher proportion in C4d+ patients. The patients were followed up for 3.5 years. Four patients in the C4d+ group evolved to ESRD in the follow-up, but none of the patients in the C4d- group ($p = 0.064$)[16].

The present study showed **C4d immune deposition** in 28 cases out of 40. 12 cases (30%) showed no c4d immune deposition.C4d 1+ was observed in 8 cases (20%), 2+ in 14 cases (35%) , 3+ in 6 cases (15%). All 28 cases showed deposition of C4d in the glomerular structures which was comparable to both the studies. No peritubular capillary c4d deposition was observed.

Reasons for the negativity in 30% of the cases in present study could be due to

1. Variable period of fixation and
2. C4d may not be that sensitive to pick up less intensity staining.

V LIMITATIONS OF THIS STUDY

Although the study sample size is not negligible, it would have been more valuable to have had an appropriate follow up to establish valid associations between disease activity and C4d deposition. Grading of C4d is subjected to personal variation.

VI CONCLUSION

C4d positivity indicates activation of insitu classical complement pathway in lupus nephritis. C4d deposition in glomerular capillaries of lupus nephritis does not indicate the present disease activity. Pattern of C4d immune deposition was coarsely granular, linear along the entire capillary circumference. Further studies are needed to evaluate C4d as a biomarker of disease activity in lupus nephritis. Further studies are also needed to explain the C4d negativity.

ACKNOWLEDGMENT

I thank all the patients for their participation, Apollo health campus team for their immense help, Osmania Medical College nephrology team for all the clinical support and my staff

REFERENCES

1. Fauci A. Braunwald E. Isselbacherk, et al.Harrison's principles of internal medicine 2000: 1922-1928.
2. Regele H, Bohmig GA, Habicht A, et al.Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. J Am Soc Nephrol. 2002; 13:2371-2380.
3. Buyon JP, Tamerius J et al. Assessment of disease activity and impending flare in patients with SLE. Comparison of the use of complement split products and conventional measurements of complement. Arthritis Rheum 1992;35:1028-37
4. Falk RJ, Dalmasso AP, Kim Y et al. Radioimmunoassay of the attack complex of complement in serum from patients with systemic lupus erythematosus. N Engl J Med 1985;312:1594-9.
5. Senaldi G, Makinde VA, Vergani D, Isenberg DA. Correlation of the activation of the fourth component of complement (C4) with disease activity in systemiclupus erythematosus. Ann Rheum Dis 1988;47:913-7
6. Gawrl MS, CHudwin DS et al. The terminal complement complex, C5b-9, a marker of disease activity in patients with systemic lupus erythematosus. Arthritis Rheum 1988;31:188-95
7. Hopkins P, Belmont HM et al. Increased levels of plasma anaphylatoxins in systemic lupus erythematosus predict flares of the disease and may elicit vascular injury in lupus cerebritis. Arthritis Rheum 1988;31:632-41
8. Porcel JM, Ordi J et al.The value of complement activation products in the assessment of systemic lupus erthematosus flares, Clin Immunol Immunopathol 1955; 74:283-8
9. Kerr LD, Adelsberg BR et al. Complement activation in systemic lupus erythematosus: amarker of inflammation. J Rheumatol 1986;13:313-9
10. Rother E, Lang B, Coldewey R et al. Commplement split product C3d as an indicator of disease activity in systemic lupus erythematosus. Clin Rheumatol 1993;12:31-5
11. Negi VS, Aggarwal A et al. Complement degradation product C3d in urine: marker of lupus nephritis. J Rheumatol 200;27:380-3
12. The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited Jan J. Weening, Vivette D. D'Agati et. al
13. Press EM, Gagnon J. Human complement component C4. Structural studies on the fragments derived from C4b b y cleavage with C3b inactivator. Biochem J 1981;199:351-7
14. Kim SH, Jeong HJ. Glomerular C4d deposition indicates in situ classic complement pathway activation, but is not a marker for lupus nephritis activity. Yonsei Med J. 2003; 44:75-80.
15. Sumanth.R.Daram et al Patterns of C4d Staining in Patients with Lupus Nephritis The Journal of Applied Research • Vol. 6, No. 1, 2006
16. Sahin OZ, Gurses S, Taslı F, Yavas H, Ersoy R, Uzum A, Cirit M. Glomerular c4d staining can be an indicator of disease activity in lupus nephritis, Ren Fail. 2013;35(2):222-5

Authors

First Author – Dr.Bheemavathi.A, MD.Pathology, Osmania Medical College, dr.bheemavathi@gmail.com

Second Author – Dr.K.Durga, MD.Pathology, Osmania Medical College, drkdurga.path@gmail.com

Third Author – Dr.Swarnalata Gowrishankar, MD.Pathology, Apollo Hospitals, swarnalatag@gmail.com

Correspondence Author – Dr.Bheemavathi.A, MD.Pathology, Osmania Medical College

dr.bheemavathi@gmail.com, 919951562670