

## INTRODUCTION

- Membranous nephropathy (MN) is one of the commonest causes of nephrotic syndrome in adults
- It is an immune complex-mediated disease characterized by deposition of antigen-antibody immune complexes in subepithelium of glomerular basement membrane (GBM)
- MN has been characterized by deposition of M-type phospholipase A2 receptor (PLA2R) and Thrombospondin type - I Domain containing 7A (THSD7A)
- Recently, a novel antigen called Neural epidermal growth factor-like protein (NELL-1) was identified in PLA2R and THSD7A negative MN cases by mass spectrometry
- Data on PLA2R-related & THSD7A-related MN in Indian literature is limited
- Role of NELL-1 in MN has been described in few studies in world literature to date
- The present study aims to profile membranous nephropathy based on the expression of NELL-1, PLA2R & THSD7A immunohistochemically

## MATERIALS & METHODS

- It was a prospective & retrospective observational study and included renal biopsies received in the Department of Pathology at AIIMS, Jodhpur over a period of 5 years
- Two cores were received for each case. One was fixed in formalin and processed for light microscopy evaluation & second core was received in normal saline and processed for immunofluorescence (IF) microscopy
- All the formalin-fixed paraffin-embedded tissue sections were stained with H&E, PAS, Trichrome, & Jones methenamine silver stains.
- IF was done against the antisera specific for IgG, IgA, IgM, Complements C3 & C1q, and Kappa and Lambda light chains
- Immunohistochemistry (IHC) for NELL-1, PLA2R, & THSD7A was put up on all cases diagnosed as MN on light and immunofluorescence
- Diffuse granular capillary wall staining of glomerular capillary walls was considered positive for all 3 stains

## RESULTS

- Of the total 474 renal biopsies received, 50 cases were diagnosed as MN
- Of the 50 cases, 47 had adequate tissue for all 3 immunohistochemical stains and were included in the study
- Median age of patients with MN was 40 years; Males=27, Females=20
- N=47, Primary MN=32 (68.08%), Secondary MN=15 (31.92%)
- One patient of primary MN exhibited triple antigen positivity, while there were no such cases in secondary MN
- 5 triple negative, 1 was primary and 4 were secondary MN
- 25/47 had only PLA2R positive, only NELL1 was positive in only 2 cases

Variables	n(%) [N=47]	
Hypertension	12 (25.50)	
Diabetes mellitus	3 (6.40)	
Systemic Lupus Erythematosus	11 (23.40)	
Hepatitis B infection	2 (4.30)	
Malignancies	0 (0.0)	
Dyslipidemia	33 (70.20)	
Proteinuria	1*	2 (4.30)
	2*	10 (21.30)
	3*	31 (66.0)
	4*	4 (8.50)
Hematuria	12 (25.50)	

Variables	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	$\chi^2$ value	p value
Mesangial hypercellularity	4 (12.5)	10 (66.7)	14.3	<0.001
Endocapillary hypercellularity	0 (0.0)	7 (46.7)	-	-
Crescent formation	0 (0.0)	2 (13.3)	-	-
Focal segment glomerulosclerosis	17 (53.1)	7 (46.7)	0.75	0.686
Arteriosclerosis	18 (56.3)	8 (53.3)	0.03	0.851
Arteriolosclerosis	12 (37.5)	5 (33.3)	0.07	0.782

	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	Total [N=47] n (%)
<b>Single antigen positives</b>			
Only NELL 1	1 (3.12)	1 (6.67)	2 (4.24)
Only PLA2R	19 (59.38)	6 (40.0)	25 (53.19)
Only THSD7A	1 (3.12)	1 (6.67)	2 (4.24)
<b>Double antigen positives</b>			
NELL1 and PLA2R	7 (21.89)	2 (13.32)	9 (19.14)
NELL1 and THSD7A	0 (0.0)	1 (6.67)	1 (2.12)
PLA2R and THSD7A	2 (6.25)	0 (0.0)	2 (4.24)
<b>Triple antigen positive</b>			
Triple antigen positive	1 (3.12)	0 (0.0)	1 (2.12)
<b>Triple antigen negative</b>			
Triple antigen negative	1 (3.12)	4 (26.67)	5 (10.63)

Antigens	Primary MN [N=32] n (%)	Secondary MN [N=15] n (%)	Total [N=47] N (%)	$\chi^2$ value	p value
NELL 1	9 (28.1)	4 (25.7)	13 (27.65)	0.01	1.000
PLA2R	29 (90.6)	8 (53.3)	37 (78.72)	8.48	0.007
THSD7A	4 (12.5)	2 (13.3)	6 (14.89)	0.00	1.000

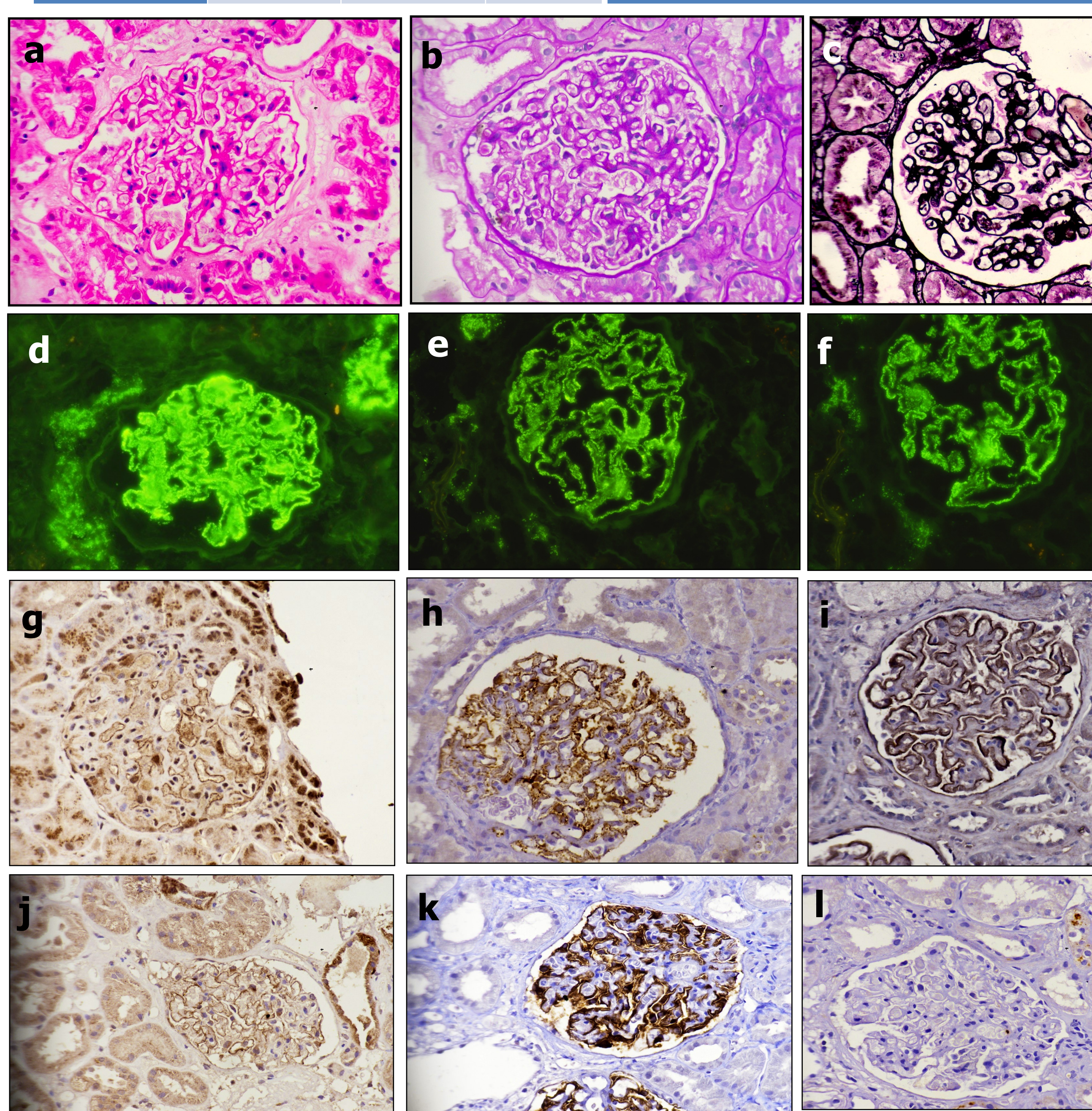


Figure 1 a& b) uniform thickening of the glomerular capillary loop; H&E x400 & PAS x400 c) JMS showing craters & spikes x400, d-f) Intense capillary wall granular staining on IF with IgG, kappa, & lambda; x200 g-i) Bright capillary wall granular staining with IHC for NELL-1 (g, x400), PLA2R (h, x400), & THSD7a (i, x400)

## DISCUSSION

- MN is broadly classified as primary and secondary depending on the cause
- Primary membranous nephropathy earlier considered to be idiopathic has now been characterized by deposition of PLA2R & THSD7A which accounts for approximately 75% and 5% of cases respectively
- NELL-1 is a gene that is strongly expressed in neural tissue encoding a protein with EGF-like repeats
- In kidney, tubules have highest expression of NELL-1

- Globally, many studies have shown that primary & secondary MN account for approximately 5-80% & 20-25% of cases respectively
- Many studies debate on strong association of malignancies with secondary MN; no malignancies were observed in the present study
- Caza et al have suggested NELL-1 as target antigen in malignancy-associated MN, No association with malignancy was seen in the NELL-1-positive MN cases in the present study
- 24-hour urinary protein levels were found to be significantly higher among patients with PLA2R positivity, similar result was seen by Subramanian P et al and Gudipati A et al
- PLA2R antigen was most commonly expressed in primary MN similar to studies by Beck et al
- Most studies suggest that NELL-1 is more commonly expressed among PLA2R-negative cases
- Wang G et al in 2021 showed t expression of NELL-1 with PLA2 in 1 case in their study
- Similarly present study showed NELL-1 & PLA2R positivity in 9 cases & NELL-1 & THSD7A positivity in 1 case
- It is believed that PLA2R & THSD7A are expressed together due to a common antigenic motif in N terminal region in these antigens that activates B cells to produce antibodies
- These antibodies may be directed against both antigens or any one of the two
- Recent studies have shown that these antigens are expressed even in secondary MN, which is also affirmed by the findings in the present study

## CONCLUSION

- Among the cases, majority showed PLA2R positivity exclusively
- Expression of NELL-1 was more common than THSD7A in the study group
- Double antigen positive were found among a quarter of patients in the study
- No definite association of NELL-1 positivity and malignancy was seen in the study
- PLA2R although considered marker for primary MN was also seen in cases of secondary MN in the present study
- Lack of statistical associations in present study cannot be considered conclusive, given the smaller sample size

## REFERENCES

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