International Journal of Advances in Nephrology Research

Volume 7, Issue 1, Page 98-103, 2024; Article no.IJANR.119059

Differences in the Clinicopathological Features of Membranous Nephropathy Associated with NELL-1 Versus PLA2R -A Single Center Experience

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/119059

Original Research Article

Received: 15/06/2024
Accepted: 17/08/2024
Published: 23/08/2024

ABSTRACT

Background: The present study discusses changing clinicopathological profile in Membranous Nephropathy(MN). MN is a disease classically presenting with nephrotic range proteinuria along with the presence of microscopic sub-epithelial deposits in glomeruli [1]. With the discovery of novel antigens like THSD7A (thrombospondin type 1 domain containing 7A), NELL-1 (neural epidermal growth factor-like 1 protein) classification of patients into primary (associated with phospholipase A-

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Cite as: *Agarwal, Divyansh, Nikita Hapani, Pankaj Beniwal, Rakesh Gupta, and Dhananjai Agarwal. 2024. "Differences in the Clinicopathological Features of Membranous Nephropathy Associated With NELL-1 Versus PLA2R -A Single Center Experience". International Journal of Advances in Nephrology Research 7 (1):98-103. https://journalijanr.com/index.php/IJANR/article/view/61.*

2 receptor antibody) and secondary membranous nephropathy has become difficult [2]. This study aims to throw light on the clinicopathological profile of patients of MN associated with NELL-1 deposits.

Methodology: Data of 52 patients with MN was reviewed and patients were followed up for 1-year post-diagnosis for the remission and development of signs or symptoms suggestive of malignancy. All the patients were given standard care as per KDIGO.

Results: 7 patients came out to be MN with NELL-1 deposition. However, a raised association of malignancy among NELL-1 positive patients was not found.

Conclusion: We conclude that NELL-1 positivity is frequently seen in the elderly age group. Patients with NELL-1 deposition have similar clinical profiles as compared to patients with PLA2R-positive patients. Moreover, the patients with NELL-1 deposits are treatment-resistant as compared to patients with PLA2R deposits. No increased incidence of malignancy was found amongst patients with NELL-1 deposits.

Keywords: NELL1; Membranous nephropathy; malignancies; IgG levels.

1. INTRODUCTION

Membranous nephropathy (MN) is a prevalent cause of nephrotic syndrome in adults and can either occur as a primary condition or be associated with various underlying etiologies characterized by unique antigens and antibody complexes localized in the subepithelial region of glomeruli [1]. Beck et al.'s identification of the phospholipase A2 receptor (PLA2R) antigen within these deposits, along with immunoglobulin, has spurred significant clinical and research advancements in primary MN. Currently, PLA2R is implicated in 70% to 80% of primary MN cases, while THSD7A is associated with 1% to 5% [1].

Recent advances using laser microdissection of glomeruli and mass spectrometry have unveiled additional antigens linked to autoimmune mechanisms in MN, such as exostosin [2]. Sethi et al. identified NELL-1 as a novel antigen in cases negative for PLA2R and THSD7A, categorizing it as a distinct cause of primary MN. NELL-1, encoding a 90-kDa protein of 810 amino acids, is highly expressed in osteoblasts and has been detected in the extracellular matrix of human embryonic kidneys, suggesting potential deposition in the glomerular basement membrane. Immunohistochemical staining has confirmed NELL-1 presence in the glomerular basement membrane and subepithelium of affected patients.

Unlike PLA2R-positive MN, where malignancies are rare and considered coincidental, the association between NELL-1 and malignancies remains unclear [3]. Initial studies did not detect tumors in patients with NELL-1-positive MN; however, whether NELL-1 positivity in some cases correlates with tumor presence, similar to THSD7A, is yet undetermined. Thus, the classification of NELL-1 as a primary MN etiology or its association with malignancies requires further investigation.

1.1 Objectives

Determination of clinicopathological profile of NELL-1 associated membranous nephropathy

2. METHODOLOGY

A retrospective study was conducted in the department of nephrology, at Sawai Man Singh Medical College Jaipur from January 2023 to December 2023 wherein data of 440 patients with renal pathology was collected. The study enrolled patients whose renal biopsy was suggestive of membranous nephropathy. However, the study excluded patients with known lupus nephritis, diagnosed cases of malignancy, or any feature suggestive of disease-causing secondary membranous nephropathy.

A thorough history was taken of the enrolled patients along with their informed consent. The patient's creatinine was measured at presentation and regularly at subsequent visits along with a dipstick (tetra bromophenol blue) for proteinuria along with 24-hour urinary protein (using spectrophotometry). Renal biopsy was performed using a 16G renal biopsy gun with the help of ultrasound by a nephrologist and samples were subsequently sent for light microscopy and immunofluorescence staining for the type of deposits was done. All patients underwent

routine investigations and were tested for viral markers including HIV, Hepatitis B, and Hepatitis C. Quantitative serum PLA2R antibody levels were also done in patients without deposition of PLA2R and THSD7A. All the patients underwent thorough workup for malignancy including chest x-ray, ultrasonography of the abdomen, complete blood count, stool routine and microscopy, and pap smear as per indication. Patients who experienced a >50% reduction in proteinuria alongside stable renal function and increased serum albumin levels were classified as being in remission, while those who did not meet these criteria were categorized as resistant. No cases of relapse and post-renal transplant were enrolled in the study. The patients were categorized as per their risk profile as suggested by KDIGO [4]. All patients underwent serum PLA2R antibody test at the initiation of treatment and if elevated after 6 months a repeat test was conducted. The patients were given treatment as per KDIGO protocols [4]. Urinary protein was checked quarterly i.e. at 0,3,6,9,12 months. Extensive data recording was kept using Excel data sheets and no breach of data privacy was ensured.

3. RESULTS

Out of 440 patients biopsied, 52 came out to be having MN with a female preponderance of 29 patients. All the patients fell into moderate and high-risk profiles as per KDIGO recommendation.

Table 1. GFR distribution of studied patients

Mean GFR(ml/min/m^2)	Number
>60	32
45-59	12
30-44	8
15-29	0
<15	0

Of these 52 patients 7(13.5%) were seen associated with NELL1 deposition in glomeruli with negative PLA2R levels in serum. The THSD7A was found positive in 3 patients while no deposition was found in 2 patients. Conservative treatment alone using ACEi/ARBs was used in 12 patients while 40 patients received immunosuppressive and conservative therapy. Immunosuppression included the use of injection of rituximab 500mg weekly for 4 weeks.

The patients were followed up for the subsequent 6 months with achievement of >50% decrease in proteinuria in 45 out of 52 patients. Serum creatinine also improved from a mean of 1.34 to 1.16mg/dl along with an increase in serum albumin from a mean of 2.3 to 2.8mg/dl. The patients with PLA2R deposits were more responsive to treatment with a decrease in mean serum creatinine from 1.3 to 1.04 at 6 months after initiation of treatment as compared to patients with NELL1 deposits where mean serum creatinine decreased from 1.4 to 1.21.

Table 2. Clinical characteristics of patients with NELL-1 and PLA2R deposits

Characteristics	NELL-1	PLA2R
Total cases	7	40
Mean age (in years)	55.4	47.8
Serum anti-PLA2R antibody	Nil	31
Sex (no. of females)	5	22
Proteinuria at baseline (g/l)	8.4	8.1
Proteinuria at 3months (g/l)	4.8	2.1
Proteinuria at 6months (g/l)	4.6	1.7
Presence of hypertension	2	23
Diabetes Mellitus	Nil	2
Mean Creatinine	1.4	1.3
Malignancy	Nil	3
Serum protein (mg/dl)	4.1	4.2
Serum albumin (mg/dl)	2.2	2.3
Mean GFR (ml/min/1.73m^2)	59ml/min/1.73m^2	68ml/min/1.73m^2
No. of patients receiving immunosuppression	7	28
Remission	4	34
Resistant	3	6
Predominant IgG subtype on IF	IgG4	IgG1

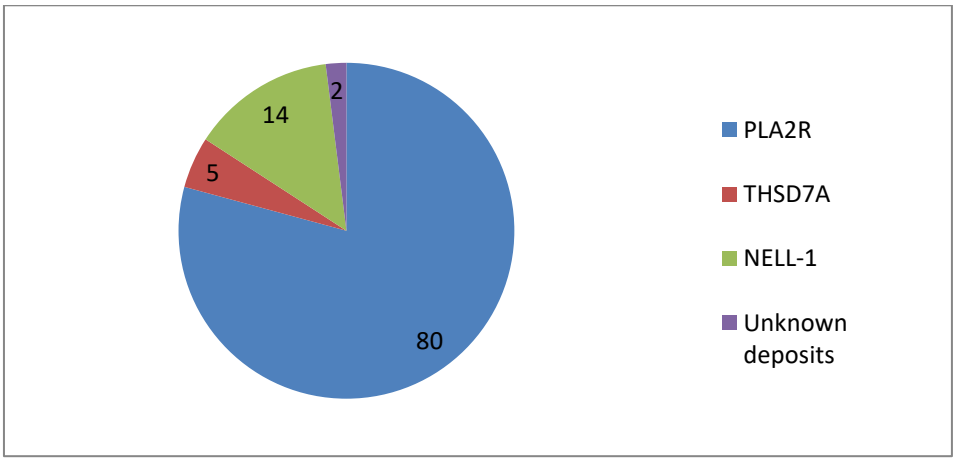

Fig. 1. Distribution as per type of deposit

Significant reduction in the amount of proteinuria was seen in cases of patients with PLA2R deposits where proteinuria at 6 months of treatment was 1.7g/l as compared to 8.1g/l at presentation. However, similar findings couldn't be reciprocated in patients with NELL1 deposits where proteinuria decreased from 8.4g/l at presentation to 4.6g/l at 6 months of therapy. Although serum albumin and total protein levels increased subsequently in both groups the difference wasn't significant

The patients were closely monitored but no signs suggestive of malignancy were found in 1 year after initiation of therapy. No patients among NELL1 deposits were found to have any autoimmune disease like autoimmune thyroiditis, Sjogren's syndrome, or sarcoidosis. None of the patients had a prolonged history of NSAID use. All the patients were negative for hepatitis B and hepatitis C. These features had previously been described as a cause of secondary deposition of NELL1 [5].

Amongst the 7 patients with NELL1 deposits 5 were female while the remaining were male. 2 patients with Nell 1 positivity had deranged renal functions with creatinine 2.4 and 2.8 subsequently. All 7 patients were treated with injection rituximab 500mg/week for 4 weeks but remission was achieved in only 4 patients while there was no improvement in serum creatinine levels. All the treatment-resistant patients were started on immunosuppressive therapy including injection cyclophosphamide and oral prednisolone. Incidentally, all 7 patients with NELL1 glomerular deposition also had IgG1 deposits as compared to patients with PLA2R deposits where predominantly IgG4 deposits were found. Using various screening methods no signs of malignancy were found in NELL1 patients however, 3 patients with PLA2R positivity were found to have malignancy. Two patients had carcinoma of the colon while one had a lung tumor that turned out to be small cell carcinoma.

4. DISCUSSION

Membranous nephropathy (MN) is a distinctive glomerular condition and the predominant cause of idiopathic nephrotic syndrome in nondiabetic adults. Approximately 80% of cases are classified as primary MN (PMN), with the remaining 20% associated with secondary MN, linked to systemic diseases or exposures [6]. Primary MN is primarily mediated by antibodies targeting the M-type phospholipase A2 receptor (anti-PLA2R) (85%), thrombospondin type 1 domain containing 7A (THSD7A) (3%–5%), or other as yet unidentified mechanisms (10%) [7,8].

Our study observed a prevalence of 76% PLA2R-positive patients and 5% THSD7A-positive patients, while 3% were attributed to unidentified mechanisms. This lower incidence of unidentified mechanisms might be attributed, in part, to the absence of testing for NELL1 deposition in previous studies. As noted by Sanjeev Sethi [9,10], recent research has identified novel types of MN associated with EXT1, EXT2, NELL1, Sema3B, and PCDH7, each representing distinct disease entities with unique clinical and pathologic characteristics. Future clinical testing for these novel agents is expected to further reduce the percentage of cases classified under unidentified mechanisms.

In parallel to the study by Nicole Andeen et. al. [11] the patients of NELL1 positivity do present with nephrotic range proteinuria with only a few presenting with decreased GFR (29%). As analogous to the study by A.G. Kattah et. al. [12] where the primary deposit was found to be IgG4 in primary MN associated with PLA2R antibodies, our study also had similar findings. The predominant deposition of IgG1 in NELL1-positive cases has also been studied previously by Tiffany N. Caza [13]. Our study coincided to the same.

In opposition to the study by Tiffany N. Caza [13], the patients in our study with NELL1 positivity did not respond adequately to the standard immunosuppressive and conservative therapy. However with the sample size so small it would be demanding to determine the treatment responsiveness of such patients.

In contrast to the study by Xiaoying Hu et. al. [3,14], our study did not find any correlation with malignancy. However, since the sample size is small other studies might differ from the findings of our study. Moreover, the follow-up of the study was only 1 year, and would be rudimentary to deny the association of NELL1 with malignancies. As quoted by Syeda Behjat Ahmad [3] “whether NELL-1 is truly a primary MN etiology or in some populations related to a malignant tumor remains to be determined.”

While most instances of NELL1-associated MN are likely to be categorized as idiopathic/primary MN [5], the authors suggest categorizing NELL1 MN as secondary MN, given its association with various secondary diseases documented in prior studies [5]. Moreover, standard immunosuppressive therapies have shown reduced efficacy in NELL1 MN

5. CONCLUSION

Based on the findings of the study it can be concluded that patients with NELL1 deposit belong to an older age group as compared to overall patients of MN. Patients with NELL1 deposit cannot be differentiated from patients of primary MN based on clinical features, nor the GFR deposit or amount of proteinuria. The patients with NELL 1 deposit also appear to be treatment-resistant, however further subsequent research is required for the treatment strategies. Moreover, it can be safely concluded that patients with NELL1 deposits do not seem to be at a higher risk for malignancies.

6. LIMITATIONS OF THE STUDY

The study was a single-center study with patients evaluated from the last 1 year only. With such a small sample size, the extrapolation of results to the general would be deafening. The association of NELL1 MN with malignancy needs long follow-up and regular evaluation. Response and relapse of NELL1 MN associated with traditional treatment of MN needs to be further instigated before any conclusion can be drawn.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/119059