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RESEARCH LETTER

The Antigenic Landscape of Pediatric Membranous Nephropathy

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INTRODUCTION

Membranous nephropathy (MN) is rare in children, comprising 3% of pediatric nephrotic syndrome.[1] MN may be underrecognized in children because the majority are empirically treated with corticosteroids for suspected podocytopathy and do not undergo biopsy. Whereas MN is well-characterized in adults, clinicopathologic characteristics and the antigenic distribution of pediatric MN is lacking, with only 1 large cohort of pediatric patients with MN reported.[2] The antigen distribution of MN in adults in the United States is different from Eastern cohorts from India and China,[3,4] likely due to a combination of genetic and environmental factors. Currently, the antigenic distribution of MN in children is yet to be investigated outside of the United States. This study represents the first large study examining MN in children in an Eastern cohort with 90 patients with MN from India.

RESULTS

Ninety pediatric patients with a diagnosis of MN were identified from the pathology archives in a 4-year period at the Renopath Center for Kidney and Urological Pathology in Chennai, India, from 2018 to 2023. Biopsies were received from 55 centers throughout Southern India.

Patients ranged from 2 to 18 years, with a mean age of 14.7 ± 3.2 years. The majority were ≥10 years (91.1%). There were 56 females (62.2%) and 34 males (37.8%). Comorbid conditions were not common, with 2 patients having diabetes and 2 with hypertension. Five had a history of indigenous medicine use (Siddha and Ayurveda, mean = 4.5 months). The majority of patients presented with nephrotic range proteinuria (62.7%) with mean urine protein-to-creatinine ratio of 7.0 ± 5.8 g/g and preserved kidney function (mean Cr = 0.76 ± 0.36 mg/dl). No patients had a history of malignancy. Twenty-three patients had positive antinuclear autoantibodies. Infectious agents, a known trigger of MN,[5-9] were not identified (including hepatitis B virus, hepatitis C virus, HIV, malaria, leprosy, or tuberculosis).

Seventy-seven patients had available biopsy tissue remaining for antigen typing (Supplementary Methods). Of 77 patients, 29 (37.7%) were phospholipase A2 receptor positive (PLA2R+), 22 (28.6%) exostosin 1 or 2 positive (EXT1/2+), 6 (7.8%) neural epidermal growth factor 1 positive (NELL1+), 1 (1.3%) semaphorin 3B positive (SEMA3B+), and 18 (23.4%) were "quadruple negative" of unknown type (Supplementary Figures S1 and S2). In addition, 1 patient with MN diagnosed at 2 years of age because of cationic bovine serum albumin, was put on a diet without cow's milk products, and went into remission.

There was a significant female predominance for patients with EXT1/2 positive MN (81.8%), NELL1 positive MN (100%), and a slight predominance in patients with "quadruple negative" antigen status (55.6%), but not in PLA2R positive patients (44.8%, EXT1/2 vs. PLA2R $P = 0.0098$; NELL1 vs. PLA2R $P = 0.02$). There were no significant differences in renal function or proteinuria between groups. Patients with EXT1/2 positive MN were more likely to have a positive antinuclear autoantibodies (78.6% vs. 15.6%, $P < 0.0001$) and to be diagnosed with lupus (42.9% vs. 4.4%, $P = 0.0014$) than EXT1/2 negative patients. Five of 6 patients with NELL1+ MN had a history of indigenous medicine use known to contain mercury and the remaining patient had a history of mercury-contaminated skin whitening cream use. There were no patients taking indigenous medicines that were

Table 1. Characteristics of pediatric patients with membranous nephropathy by antigen type

Antigen type	*N*	Age (yrs; range)	% F/% M	UPCR (± SD) g/g	Cr (± SD) mg/dl	Native medicine	HTN	DM
PLA2R	29	16.0 (10–18)	44.8% F 55.2% M	6.9 ± 6.8	0.8 ± 0.5	0/29	0/29	0/29
EXT1	22	14.7 (8–18)	81.8% F 18.2% M	10.0 ± 6.8	0.8 ± 0.4	0/22	0/22	0/22
NELL1	6	14.8 (14–17)	100% F 0% M	4.8 ± 3.7	0.7 ± 0.2	5/6	0/6	0/6
SEMA3B	1	6	M	8.8	0.4	0/1	0/1	0/1
Cationic BSA	1	2	M	NA	NA	0/1	0/1	0/1
Quadruple negative	18	14.0 (6–18)	55.6% F 44.4% M	6.2 ± 4.1	0.7 ± 0.2	0/18	2/18	2/18

BSA, bovine serum albumin; DM, diabetes mellitus; EXT1, exostosin 1; F, female; HTN, hypertension; M, male; N, number of patients; NELL1, neural epidermal growth factor 1; PLA2R, phospholipase A2 receptor; SEMA3B, semaphorin 3B; UPCR, urine protein-to-creatinine ratio.

NELL1-negative (NELL1+ vs. NELL1−, $P < 0.0001$). Upon cessation of the underlying trigger, all NELL1 positive patients had reduced proteinuria (mean proteinuria at time of biopsy 4.8 ± 3.7 g/d compared to 0.8 ± 1.2 g/d at follow-up, median follow-up = 4.5 months). In "quadruple negative" MN, 26.7% were antinuclear autoantibodies+ with 13.3% having lupus. There were no patients with drug exposure, malignancy, or infection in "quadruple negative" patients. Patient characteristics of antigen groups are shown in Table 1.

Clinical follow-up was available from 59 patients (65.6%) with a median interval of 19 months (range = 2–63). Fifty-seven patients (96.6%) achieved at least partial remission (mean proteinuria 1.0 ± 1.4 g/g with proteinuria reduction of 5.7 ± 5.6 g/g). The 2 patients who did not achieve remission were untreated. Nearly all patients had normal renal function at follow-up. Two patients had disease relapse and 1 developed progressive disease and underwent transplantation.

Nearly all patients with available follow-up (96.6%) received 1 or more forms of treatment with 16 patients (27.1%) receiving ACE inhibitors/angiotensin receptor blockade and 42 (71.2%) receiving immunosuppression. Seventeen patients (28.8%) were treated with more than 1 agent, 9 of which included the Ponticelli regimen. Of the 42 patients on immunosuppression, 30 received glucocorticoids, 15 received calcineurin inhibitors, and 6 had rituximab. When comparing antigen types, there was a similar frequency of immunosuppressant use, except for NELL1+ patients treated by cessation of an underlying trigger (Table 2).

DISCUSSION

We present a large case series detailing the antigenic distribution of childhood MN and the first cohort from an Indian population. Pediatric MN is rare in children, and therefore, there is limited information on histopathologic features and clinical management, particularly outside of the United States.

The most common antigen in MN, PLA2R, had a much lower frequency than the prevalence reported in adults (37.7% children, compared to 60%–70% PLA2R+ in adults).[S1] Lower rates of PLA2R positivity in children was observed before, with a frequency of 32% to 45% in 3 cohorts.[2,S2,S3] SEMA3B was reported to be a dominant antigen in childhood MN and comprise approximately 10% of pediatric MN cases in the United States, Italy, and France.[S4] Only a single patient was SEMA3B-positive in our series, which may suggest a lower frequency of SEMA3B positive MN in India.

Interestingly, there was an increased frequency of EXT1/2 positive MN compared to that reported in adults.[S5,S6] EXT1/2 positivity is common in the setting of membranous lupus nephritis,[S7] and there was an increased frequency of patients found to have lupus at follow-up. EXT-positivity may be a harbinger of underlying autoimmune disease and we advise that these patients have thorough rheumatologic follow-up for underlying autoimmunity.

Indigenous medicine use was common in patients with NELL1 positive MN, an association similarly identified in adult MN.[4] Two were treated for bronchial asthma, 1 for arthritis, and the reason for treatment was unknown in 2 patients. Previous work from our group identified that indigenous medicines contain mercury, with mercury poisoning being the likely etiology of MN.[4,S8] The 1 NELL1 positive patient without indigenous medicine use used skin whitening cream with elevated levels of mercury. Use of skin whitening creams have been reported to be associated with development of MN due to mercury toxicity in a Chinese cohort[18] and has recently been described in association with NELL1-positive MN.[S10] All NELL1-positive patients were adolescent girls, with female predominance likely related to environmental factors, though sex, hormonal, or genetic differences cannot be excluded.

A considerable proportion of patients with MN (23.4%) were "quadruple negative" of unknown

Table 2. Treatment and outcomes of pediatric membranous nephropathy patients ($n = 59$)

Antigen	N	ANA	ACE/ARB	IS	F/U Cr	F/U Prot	Prot ≤ 1g	CR
PLA2R	23	2 (8.7%)	4 (17.4%)	18 (78.3%) Steroids 11/18 Rituximab 5/18 CNI 7/18 Combination 9/18	0.8 ± 0.2	1.4 ± 1.6	12 (52.2%)	8 (34.8%)
EXT1	14	11 (78.6%)	2 (14.3%)	11 (78.6%) Steroids 10/11 Rituximab 0/11 CNI 5/11 Combination 5/11	0.7 ± 0.2	0.6 ± 0.8	11 (78.6%)	6 (42.9%)
NELL1	6	1 (16.7%)	4 (66.7%)	2 (33.3%) Steroids 2/2 Rituximab 0/2 CNI 0/2 Combination 0/2	0.8 ± 1.2	0.7 ± 0.1	4 (66.7%)	4 (66.7%)
SEMA3B	1	0	0	1-steroids		4.9	1	0
Quadruple negative	15	4 (26.7%)	6 (40.0%)	10 (66.7%) Steroids 6/10 Rituximab 1/10 CNI 3/10 Combination 3/10	0.7 ± 0.1	0.3 ± 0.6	12 (80.0%)	11 (73.3%)
Total	59	18 (30.5%)	16 (27.1%)	42 (71.2%) Steroids 30/42 Rituximab 6/42 CNI 15/42 Combination 17/42	0.7 ± 0.2	0.9 ± 1.4	40 (68.0%)	29 (49.2%)

ACE/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker use; ANA, anti-nuclear autoantibodies; CNI, calcineurin inhibitor; EXT1, exostosin 1; F/U, follow-up; IS, immunosuppression; N, number of patients; NELL1, neural epidermal growth factor 1; PLA2R, phospholipase A2 receptor; SEMA3B, semaphorin 3B.

antigen type. This is a higher frequency than reported in adults, with approximately 15% negative for these antigens**.** Mass spectrometry has been used for antigen discovery for MN and further studies are needed in pediatric MN to identify remaining inciting autoantigens.

CONCLUSION

In a large cohort of pediatric patients with MN in India, there was a lower frequency of PLA2R+ MN, increased EXT1/2+ MN, and an increase in the proportion of cases where the antigen remained unknown compared to adult cohorts. NELL1 positivity was seen in only patients with environmental triggers. Outcomes were favorable, with most patients having <1 g proteinuria at follow-up.

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary References.

Figure S1. Antigenic distribution of pediatric membranous nephropathy ($n = 77$ patients).

Figure S2. Representative staining of positive cases.

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