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Anti-phospholipase A2 receptor antibody in membranous nephropathy; an Indian experience

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ABSTRACT

Introduction: Autoantibodies against phospholipase-A2 Receptor (PLA_2R), a normally occurring antigen presented on podocyte membranes, have recently been implicated in the pathology of membranous nephropathy (MN).
Objectives: In this observational study, we have evaluated the correlation of patient's disease activity against serum levels of anti-PLA_2R in patients with primary and secondary MN in a North Indian cohort.
Patients and Methods: We measured serum anti-PLA_2R antibody by ELISA in 63 adult patients with MN. Out of these 63 patients, the majority (58) had primary MN (pMN) and the remaining five had secondary MN (sMN).
Results: Around 55.2% had detectable anti-PLA_2R autoantibodies (63% in those with new-onset pMN, 67% in patients with relapse and 3% in patients in remission). However, all patients with refractory pMN (n = 4) were anti-PLA_2R negative. There was significantly higher anti-PLA_2R positivity in active stage of disease in contrast to those in remission ($P < 0.001$). Autoantibody level was proportional to the disease activity, with a trend towards significance ($P = 0.052$). Hypoalbuminemia and proteinuria were also significantly worse in the anti-PLA_2R positive patients. All patients with sMN tested negative for anti-PLA_2R autoantibodies.
Conclusion: PLA_2R is a major target antigen in Indian population with idiopathic MN. Anti-PLA_2R positivity is a specific tool to detect idiopathic MN, and its levels correspond well with other disease activity markers. In addition, anti-PLA_2R antibody was negative in refractory pMN, which suggests the existence of additional autoantibodies in this subgroup, with a different target antigen. These autoantibodies may be resistant to the currently recommended immunosuppressants for the disease, thus the refractory nature of the disease.

Original

Implication for health policy/practice/research/medical education:
In an observational study,a group of patients with membranous nephropathy showed correlation of anti-PLA2R antibodies with disease activity and presence of primary MN.
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Introduction

Membranous nephropathy (MN) is amongst the most common aetiologies of nephrotic syndrome in adults, particularly in the non-diabetic population (1-3). In majority, almost three-quarter cases, MN is idiopathic or primary (pMN), however in remaining patients, it occurs secondary to a variety of conditions including hepatitis B, systemic lupus erythematosus (SLE), thyroiditis, malignancies and drugs like gold, penicillamine and captopril (4,5). Based on the rat model of Heymann's nephritis, autoimmune mechanisms were implicated in the genesis of MN (6,7). In 2009, Beck et al in their landmark study reported M-type phospholipase A_2 receptor (PLA_2R), on glomerular podocytes, to be the target antigen in 26 of their 37 (70%) patients having idiopathic MN (iMN). The circulating anti-PLA_2R antibodies target a specific region of the PLA_2R protein and are predominantly of the IgG4-type (8-11). Similar

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results with prevalence of anti-PLA$_2$R antibodies ranging from 60%-80% in idiopathic MN have been observed by different researchers worldwide (12-17). The anti-PLA$_2$R antibodies have been associated with high specificity and sensitivity of approximately 95% and 75% respectively for diagnosis of pMN. Few recent studies have also reported correlation of anti-PLA$_2$R levels with the clinical severity, with higher spontaneous remission rates observed in patients and with lower antibody titres. They can therefore be considered as a non-invasive marker for both diagnosis and prognosis in iMN (14-19). The US Food and Drug Administration (FDA) in 2014, accepted two commercially available tests for anti-PLA$_2$R autoantibodies, including an enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IIFA).

Objectives

In this observational study from a tertiary institute in North India, we have evaluated the correlation of patient's disease activity with serum anti-PLA$_2$R levels in primary and secondary forms of MN with variable levels of disease activity.

Patients and Methods

Patients

Adult patients ≥18 years of age, with biopsy-proven MN, including newly diagnosed secondary MN were prospectively enrolled over a period of 12 months, from January 2015 to December 2015.

Majority of the patients were started on angiotensin-converting-enzyme inhibitor (ACE inhibitor) or angiotensin II receptor blockers (ARBs). Decision to initiate immunosuppressive therapy (IST) was made in the follow-up, depending on the trending levels of 24-hour urinary-protein and serum creatinine (according to KDIGO guidelines 2012). Baseline characteristics of all the patients were recorded at the beginning of the study. All patients were worked up for any underlying secondary cause of MN, which included assessment of viral markers, ANA, anti-dsDNA, C3, C4, chest X-ray, PSA in men, breast examination in women, stool for occult blood, history of any implicated drugs. Patients were accordingly labelled as having pMN or sMN. 'New-onset disease' was defined as patients with having <6 months of diagnosis and no previous exposure to immunosuppressive therapy. Remission, relapse and refractory disease were defined according to the KDIGO 2012 definitions (20). Clinical data was prospectively collected.

Quantification of circulating anti-PLA$_2$R

ELISA kit (EUROIMMUN AG, Lubick, Germany) containing PLA$_2$R1-coated microplates was used to determine the circulating anti-PLA$_2$R antibody levels. After incubation with human sera, diluted 1:100 in sample for 30 minutes, antibodies were detected with anti-human IgG horse-radish peroxidase conjugate (EUROIMMUN AG) diluted 1:1000 in sample buffer for 30 minutes. Chromogen substrate solution (EUROIMMUN AG) was added for 15 minutes after washing. Stopping solution was thereafter used to stop the reaction. An automated microplate, absorbance reader (iMark, Bio-Rad, Veenendaal, the Netherlands) was used to read optical density. PLA$_2$R levels of >20 RU/mL were considered to be positive, <14 were taken as negative and levels between 14 and 20 RU/mL were taken as borderline as per the manufacturer specifications.

Ethical consideration

The study has been approved by the Institute Ethical Committee of Sanjay Gandhi Post Graduate Institute of Medical Sciences. Written informed consent was obtained from all patients. All participating patients gave written informed consent prior to inclusion in the study.

Statistical analysis

Continuous variables were presented as the mean ± SD. Frequencies or percentages were used to describe categorical variables. Differences in continuous and categorical variables were assessed using the independent sample *t* test/Mann-Whitney U test and chi-square / Fisher's exact test, respectively. SPSS version 20 for Windows (IBM, Chicago, Ill., USA) was used for statistical analysis.

Results

Primary versus secondary MN

Amongst 63 patients with MN in our study, 58 patients (92%) had pMN, while 5 patients (8%) had sMN. Amongst patients with sMN, 4 patients had SLE and 1 had hepatitis B infection. Majority of patients with pMN were males (M:F = 2.4), whereas all the patients with sMN were females (SLE being the predominant cause). Mean age of patients with iMN and sMN were 39 years and 32 years respectively. Nearly 57% of patients of iMN (n = 33) had already been initiated on immunosuppressive therapy (IST) at the time of inclusion in the study (modified Ponticelli regimen or calcineurin inhibitors or both), whereas only 1 patient (20%) with sMN had already been put on IST for lupus nephritis. All patients with sMN tested negative for anti-PLA$_2$R despite having slightly more severe disease, with higher degree of proteinuria (3656 mg/day versus 3484 mg/day, respectively) and more severe hypoalbuminemia (2.52 g/dL versus 3.37 g/dL in sMN and pMN respectively (Table 1).

Patient characteristics in primary membranous nephropathy

Half (n = 29 (50%)) of the patients with pMN had active disease at the start of study. One-third of our study population were newly diagnosed and had not received any treatment. Six patients (10%) were with disease relapse and 4 (7%) with refractory disease. The remaining 29 (50%) had responded to treatment and were under remission.

Amongst the newly diagnosed cases of pMN, 12 cases (63%) were anti-PLA$_2$R positive. Similarly, 4 (67%) with

Table 1. Primary versus secondary membranous nephropathy

	pMN	sMN
Subject, No. (%)	58 (92)	5 (8)
Male, No. (%)	41 (71)	0 (0)
Age (y) (mean ± SD)	38.9 ± 12.7 (18–63)	31.8 ± 12.6 (18–50)
IST, No. (%)	33 (57)	1 (20)
24-Hour urinary protein (mg) (mean ± SD)	3484 ± 3968	3656 ± 2330
Serum albumin (g/dL) (mean ± SD)	3.37 ± 0.88	2.52 ± 1.43
Serum creatinine (mg/dL) (mean ± SD)	1.37 ± 1.14	1.04 ± 0.43
PLA_2R positivity (%)	55.2% (with active disease)	0% (with active disease)

Abbreviations: pMN, Primary membranous nephropathy; sMN, Secondary membranous nephropathy; IST, immunosuppressive therapy.

relapse had positive values of PLA_2R antibodies (Table 2). However, none of the patients who were refractory to treatment had PLA_2R reactivity (Tables 3 and 4). Only 4 % of those in remission were PLA_2R positive (Table 2).

For further analysis, we grouped the pMN patients with active disease together (those with persistent nephrotic proteinuria, relapse of disease after achieving remission and refractory disease) and compared them to the cohort in remission. The anti-PLA_2R was significantly more positive in the active pMN group (55.2% in pMN versus 3% in sMN) ($P<0.001$) and the anti- PLA_2R levels also had a trend towards significance (103 ± 275 RU/mL in pMN versus 2 ± 5 RU/ml in sMN) ($P=0.05$).

Correlation of clinical activity in pMN patients with anti-PLA2R levels

The anti-PLA_2R levels correlated with the disease activity. Those with anti-PLA_2R positivity, had significantly lower levels of serum albumin and higher levels of proteinuria ($P<0.01$) (Figure 1). However there was no significant difference in serum creatinine and cholesterol levels among the groups. Anti-PLA_2R positivity was also directly proportional to age, with the mean being 45 years in the PLA_2R positive group as compared to 36 years in the other group ($P=0.01$; Table 5).

Discussion

This study was designed to validate PLA_2R antibody trends in North Indian patients with MN. Amongst the iMN patients, we found anti-PLA_2R positivity in 55.2% of patients with active disease, in 63% of the newly diagnosed untreated patients, and in 67% with full-blown relapse of disease. This prevalence of anti-PLA_2R is in concordance with other studies which have

Table 2. Baseline characteristics of patients with primary membranous nephropathy

Patient characteristics	Subtypes of pMN			
	New (Treatment-naïve)	Relapse	Refractory	Remission
Subject, No. (%)	19 (33)	6 (10)	4 (7)	29 (50)
Male, No. (%)	17 (90)	5 (83)	3 (75)	16 (55)
Age (y) (mean ± SD)	41 ± 14 (18 – 63)	41 ± 14 (18 - 56)	29 ± 15 (18 - 50)	39 ± 11 (18 – 58)
24-Hour urinary protein (mg) (mean ± SD)	6991 ± 4531	5175 ± 2954	3910 ± 1094	778 ± 778
Serum creatinine (mg/dl) (mean ± SD)	1.33 ± 0.61	0.95 ± 0.29	1.84 ± 1.5	1.42 ± 1.44
Serum albumin (g/dL) (mean ± SD)	2.69 ± 0.94	3.47 ± 0.69	3.02 ± 0.7	3.84 ± 0.54
PLA_2R positive, No. (%)	12 (63)	4 (67)	0 (0)	1 (3)
Anti-PLA_2R (RU/mL) (mean ± SD)	143 ± 335	41 ± 37	6 ± 6	2 ± 5

Abbreviations: pMN, primary membranous nephropathy.

Table 3. Comparison of pMN patients as per disease activity

Patient characteristics	Subtypes of pMN		
	With active disease (new + relapse + refractory)	With remission	*P* value
Subject, No. (%)	29 (50)	29 (50)	
Male, No. (%)	25 (86)	16 (55)	0.09
Age (y) (mean ± SD)	39 ± 14 (18 – 63)	39 ± 11 (18 – 58)	0.86
24-Hour urinary protein (mg) (mean ± SD)	6190 ± 4035	778 ± 778	<0.001
Serum creatinine (mg/dL) (mean ± SD)	1.32 ± 0.75	1.42 ± 1.44	0.756
Serum albumin (g/dL) (mean ± SD)	2.90 ± 0.90	3.84 ± 0.54	<0.001
PLA_2R positive, No. (%)	16 (55.2)	1 (3)	<0.001
Anti-PLA_2R (RU/mL) (mean ± SD)	103 ± 275	2 ± 5	0.05

Abbreviations: pMN, primary membranous nephropathy.

Table 4. Characteristics of primary membranous nephropathy patients with refractory disease

Age (y)/gender	Disease duration (y)	Proteinuria (mg/d)	Serum Albumin (g/dL)	Serum creatinine (mg/dL)	Anti- PLA$_2$R (RU/mL)	IST received
21/M	3	5400	3.0	1.10	6.06	P + CNI + MMF
50/M	8	2800	3.7	4.10	14.5	P + CNI
26/F	4	3540	2.0	1.08	0.00	P + CNI + Ritu
18/M	4	3900	3.4	1.10	3.96	P + CNI

Abbreviations: IST, immunosuppressive therapy; P, modified ponticelli regimen; CNI, calcineurin Inhibitor, MMF, mycofenolate mofetil; Ritu, rituximab.

Table 5. Clinical characteristics of patients with idiopathic membranous nephropathy according to anti-PLA$_2$R reactivity

Anti-PLA$_2$R antibody	Negative (n = 41)	Positive (n = 17)	*P* value
Male, No. (%)	28 (68)	13 (76)	0.53
Age at diagnosis (y)	36	45	0.01
24-Hour Urinary protein (mg/d)	2181 ± 3520	6466 ± 3394	<0.001
Urinary protein ≥ 3.5 g/d, No. (%)	7 (17%)	15 (88%)	<0.001
Serum albumin (g/dL)	3.67 ± 0.64	2.66 ± 0.97	<0.001
Serum albumin (<3.5 g/dL), No. (%)	14 (34)	14 (82)	<0.001
Serum creatinine (mg/dL), mean ± SD	1.47 ± 1.38	1.19 ± 0.47	0.42
Serum cholesterol (mg/dL), mean ± SD	217.66 ± 87.7	271.8 ± 136.6	0.08

reported seropositivity ranging from 52%-82% (19-23). In another study of 114 newly diagnosed pMN patients from India, Ramachandran et al found, 66.7% anti-PLA$_2$R positivity (by ELISA) (24). Lower prevalence in our study may be explained by the fact that we included patients with relapse and refractory disease in our study group. The anti-PLA$_2$R negativity in these patients can be explained by the possible presence of other target antigens implicated in pathogenesis of idiopathic MN such as non-PLA$_2$R podocyte antigens (25). Anti-PLA$_2$R was negative in five patients with secondary MN, majority with SLE as the secondary cause. This is consistent with the current data of anti-PLA$_2$R antibody's specificity for idiopathic MN (8,12-17,26).

Amongst subjects in clinical remission, PLA$_2$R was detectable in just one of 29 patients (3%). In contrast, 4 of 6 patients (67%) with relapse had positive PLA$_2$R antibodies, thus further corroborating the fact that the levels of PLA$_2$R reflect disease activity. Previous studies have also shown that, the antibody titres disappear with remission and reappear with disease relapses (8,13,14,17,18,27-32). Moreover, anti-PLA$_2$R levels were in direct proportion to proteinuria and serum albumin levels.

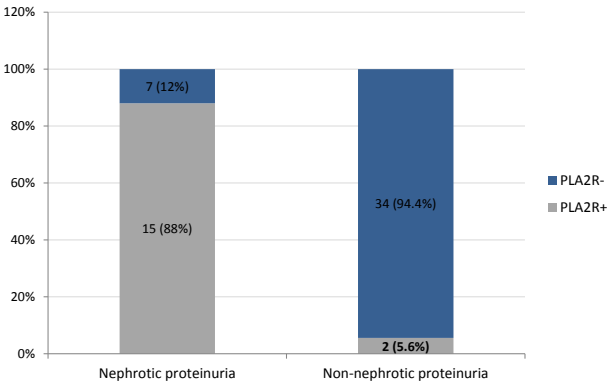

Figure 1. Prevalence of nephrotic-proteinuria in idiopathic membranous nephropathy according to PLA2R activity.

Absence of the PLA$_2$R antibodies in the four treatment-refractory patients is suggestive of presence of antibodies to some unidentified antigens, leading to a more resistant variant of the disease. Thus, the aforementioned subgroup requires further genetic and autoimmune evaluation to look for these yet unidentified autoantibodies.

No significant difference was observed in the serum creatinine levels between PLA$_2$R-positive patients and those with undetectable levels of these antibodies. Males were affected more than females (M: F = 2.4). Majority of our patients were in their third or fourth decade of life. These findings are in consistent with those reported in the literature (33).

Conclusion

To conclude, our data adds weight to the fact that anti-PLA$_2$R is a relatively sensitive and specific marker of idiopathic MN in Indian population. Furthermore anti-PLA$_2$R positivity and levels correlate with disease activity, with more severe proteinuria and lower serum albumin levels in antibody positive patients. Absence of anti-PLA$_2$R in patients with refractory pMN needs further validation and could be suggestive of more severe form of disease with different immune mechanism. Thus, anti-PLA$_2$R positivity by ELISA could possibly replace kidney biopsy in patients with suspected MN, and therefore may help to diagnose and predict the disease activity.

Limitations of the study

Our study had certain limitations. We had a relatively small sample size. Our study was confined to patients

from Northern Indian belt. In patients in remission, we did not have the baseline anti-PLA_2R levels when they had proteinuria, and there was lack of follow-up levels of anti-PLA_2R antibody

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Authors' contribution

Design and concept; MY and RKS. Data analysis; MY. The writing of the manuscript; MY, RKS, SM, NP, AG, AK and DSB. Data collection; MY and SM. Critical revision and finalizing paper; RKS. All authors read and signed the final paper.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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