Clinical implications of serum anti-PLA2R levels and glomerular PLA2R deposits in primary membranous nephropathy

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ABSTRACT

Introduction. The clinical implications of serum anti-PLA2R with glomerular PLA2R deposits in primary membranous nephropathy (PMN) is scarcely reported. Hence the study was designed to demonstrate the prevalence of serum anti-PLA2R levels and PLA2R staining in glomeruli in PMN and the clinical implications of the two parameters.

Objectives.

1. Investigate the prevalence of anti PLA2R positivity in PMN.
2. Ascertain correlation between serum anti-PLA2R levels and glomerular staining for PLA2R with clinical and lab parameters in PMN.

Patients and Methods. Fifty PMN patients during the period from October 2017 to December 2018 were included. Labs were done and eGFR was calculated as per MDRD 6. Anti-PLA2R titres were done in all patients. Titres more than 20 RU/ml were considered positive. Glomerular staining for PLA2R was graded on fresh frozen tissue by immunofluorescence technique.

Results. Anti-PLA2R antibody positivity and glomerular PLA2R deposition was observed in 42% (21/50) and 86% (43/50) patients respectively. 79.3% (23/29) had positive glomerular PLA2R deposition with negative serum anti PLA2R. Positive correlation were observed between serum PLA2R antibody and serum creatinine ($p = 0.0001$) and urine protein-creatinine ratio levels with tissue PLA2R staining grades ($p = 0.04$). Negative association was found between serum albumin ($p = 0.026$) and tissue PLA2R staining grades.

Conclusion. Serum anti-PLA2R wasn't a sensitive marker of primary membranous nephropathy in our study group emphasising the need to consider a compendium of serological markers for diagnosis of primary membranous nephropathy and to rely more on glomerular deposition of PLA2R as a better clinical indicator for PMN.

KEYWORDS: anti-PLA2R, Membranous Nephropathy, Glomerular PLA2R deposits, Tissue PLA2R staining, Nephrotic Syndrome

Introduction

Membranous nephropathy (MN) is an important aetiology of adult onset nephrotic syndrome which is subclassified into primary (PMN) and secondary membranous nephropathy. Secondary membranous nephropathy is implicated in clinical scenarios such as cancer, autoimmune diseases and infections [1, 2]. PMN can be diagnosed on the basis of biomarkers like Anti PLA2R levels which can be useful in adjusting the therapeutic initiatives for management of the disease process. These biomarkers may be used to predict clinical consequences like decreased eGFR or proteinuria [3]. The discovery of phospholipase A2 receptor (PLA2R) antibody has contributed to an improvised understanding of the pathophysiology of PMN [4]. The specificity and sensitivity of PLA2R antibody for the PMN has been approximated to be around 100% [5] and 50% to 80% respectively [6]. Previous studies have tried to assess the utility of antibodies to PLA2R in clinical practice. However, there is a definite need for more studies to study the prevalence of glomerular PLA2R deposits, so that it can be applied as a diagnostic and prognostic test in the patients with PMN.

Patients with serum anti-PLA2R often have positive PLA2R deposition in the glomeruli [7]. Even though the clinical implication of serum anti-PLA2R levels has been well established by different research teams [8, 9], the correlation of serum anti-PLA2R levels with PLA2R deposits in glomeruli, and the association between PLA2R deposits with clinical, demographic and lab parameter have been explored sparsely in medical literature. Hence this study was designed to investigate the prevalence of anti PLA2R positivity in PMN and ascertain correlation between serum anti PLA2R levels and glomerular staining for PLA2R with clinical and lab parameters in PMN.

Methodology and study design

This was a prospective observational study done in collaboration with Nephrology and Pathology department in a tertiary care hospital from October 2017 to December 2018 in accordance with existing laws governing research including the revised declaration of Helsinki (2008). Ethical clearance from the institutional ethical committee and written informed consent from participants was obtained. The sample size was deduced to be 50 patients based on a similar study done by Gopalakrishnan et al. [8] where prevalence of serum anti-PLA2R antibody in patients of PMN was found to be around 75% with a power of 80%. The enrolled participants were of South Asian ancestry, above 18 years of age, diagnosed of primary membranous nephropathy on the basis of serum anti PLA2R, clinical history (ruling out Native medication intake and tropical illnesses), lab parameters (Negative Hepatitis B and anti-Hepatitis C virus serology, negative ANA, negative stool for occult blood, negative mammogram for breast malignancy for females aged greater than 40 years, normal prostate specific antigen assay for males with age greater than 50 years and normal complement levels) and renal histology strongly suggestive of PMN (i.e. GBM thickening with granular capillary wall IgG staining in the absence of mesangial/endocapillary hypercellularity, absent mesangial IgG staining and absent C1q).

Anti-PLA2R antibody titre was measured in all enrolled study patients of PMN by ELISA methodology. Titres more than 20 RU/ml were considered positive. The renal biopsy tissue was processed for light microscopy (Jones Methenamine silver stain, Haematoxylin & Eosin and Periodic Schiff stain (Figure1 and Figure 2) with immunofluorescence and documented primary membranous nephropathy on renal biopsy was subjected to additional glomerular staining with polyclonal anti PLA2R.

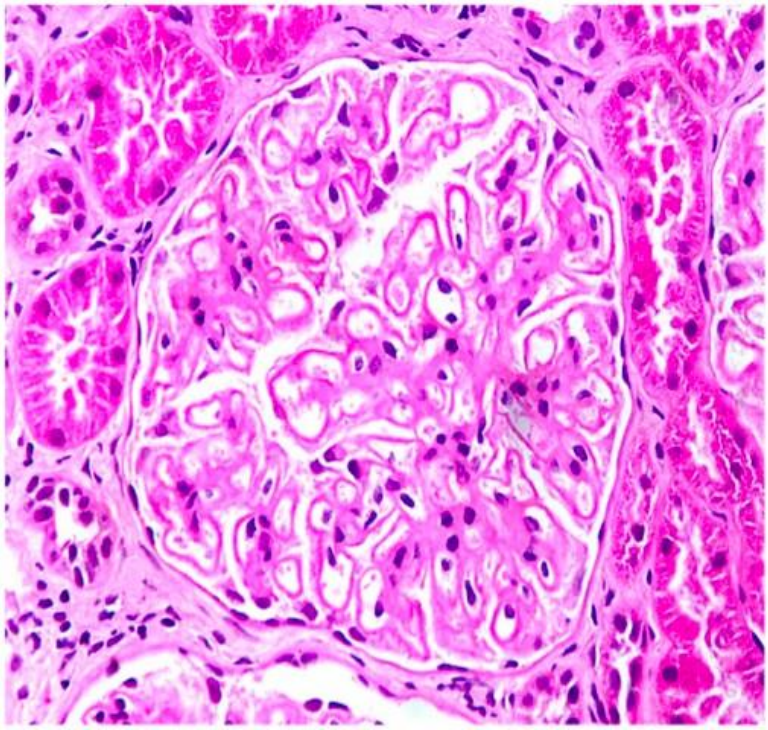

Figure 1. Light Microscopy (Periodic Schiff stain) of Primary Membranous Nephropathy. (Magnification:400x).

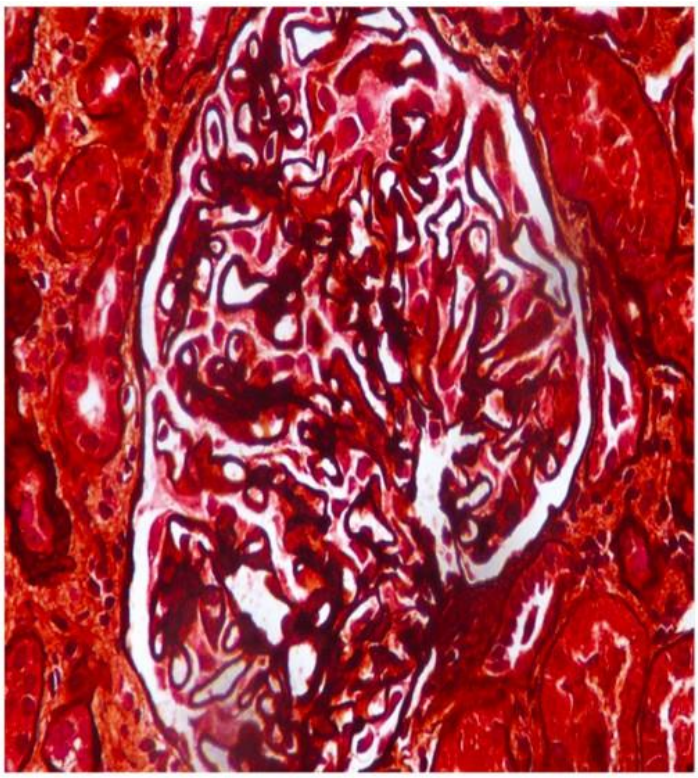

Figure 2. Jones Methenamine silver staining of Primary Membranous Nephropathy. Magnification: 400x.

Glomerular staining for PLA2R was graded by immunofluorescence technique on fresh frozen tissue after fixing initially with rabbit polyclonal anti-PLA2R antibody and later fixed with Alexa Fluor 488 goat anti-rabbit IgG. PLA2R staining was graded semi-quantitatively according to the intensity assessed by a single consultant pathologist. They were graded on a scale from 0 to +4 and staining > +2 were considered positive (Figure 3).

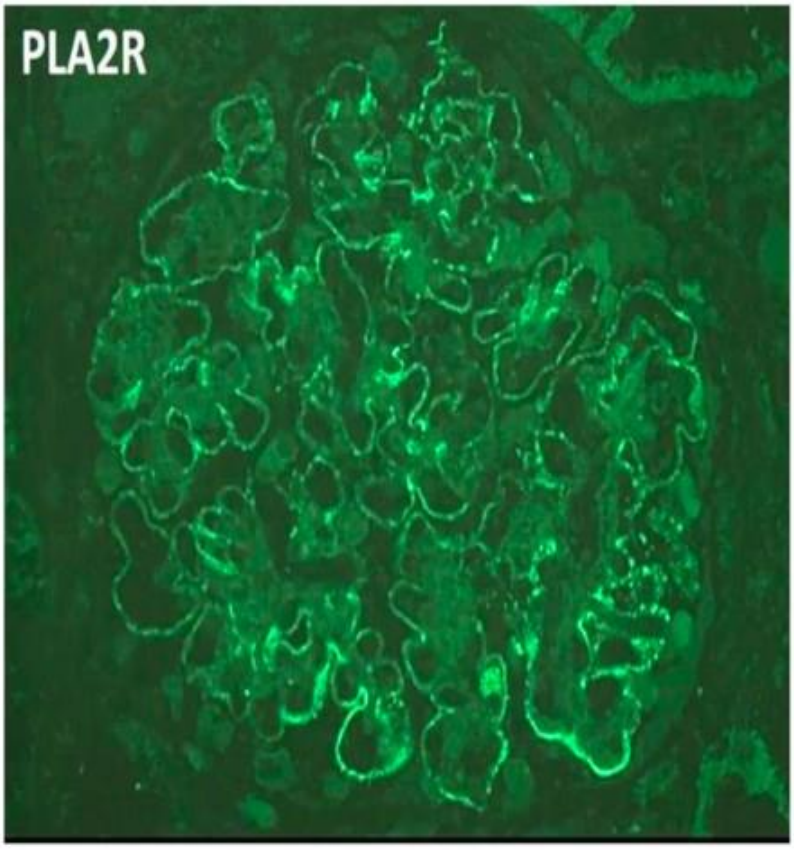

Figure 3. PLA2R staining of Glomeruli in Primary Membranous Nephropathy. Magnification: 400x.

Serum creatinine, urea, albumin, cholesterol, urine routine analysis, proteinuria quantification by urine protein creatinine ratio (UPCR) were performed 1 week prior to the renal biopsy specimen evaluation. Modification of diet in renal disease (MDRD) 6 variable formula was used for calculation of estimated Glomerular Filtration Rate (eGFR). The patients in the study group weren't exposed to Angiotensin converting enzyme inhibitors/angiotensin receptor blockers or any immunosuppressive medications at the time of biochemical assessment.

The corelation between the serum anti PLA2R levels, PLA2R staining on the glomerular deposits and concurrent correlation between PLA2R biomarkers and clinical and laboratory parameters were evaluated in this research. Nephrotic Syndrome was defined a clinical condition characterized by oedema, hypoalbuminemia (<2.5 gm/dl), proteinuria (> 3.5 gm/day/ Urine PCR>3.5 mg/mg of creatinine), and nephrotic range proteinuria was defined as: > 3.5 gm/day of proteinuria/ Urine PCR>3.5 mg /mg of creatinine) [9].

Statistical analysis

Data was processed and analysed using the software SPSS version 22.0. The continuous variables were expressed as mean $\pm$ SD or represented by median (Interquartile range). Continuous normally distributed variables were statistically assessed by independent sample 't' test or ANOVA. Categorical variables were statistically measured by Chi-square test /Fisher's exact test. The correlation between bivariate variables was statistically evaluated by Pearson's correlation coefficient. MS-Excel spreadsheet was utilized for data entry and p value < 0.05 was considered significant.

Results

Clinical details and Biochemical Parameters

Fifty patients with PMN of South Asian lineage were included. Total 928 biopsies were done during the study period of 2017-2018. PMN constituted around 5.38 % (50/928) of the patients undergoing native renal biopsy. The mean age of the patients was 45.98 ± 10.67 years. The median age of the study participants was 44.5 years (range: 17-67 years). Majority of the patients were males (74%). Table 1 describes the demographic details of the study volunteers. The various biochemical and clinical parameters included were urine protein-creatinine ratio, serum creatinine, serum urea levels, serum BUN, serum albumin and eGFR. Table 2 illustrates the mean values of these biochemical parameters measured.

Mean age (years)	45.98 ± 10.67
Median age (years)	44.5 (Range: 17-67)
Number of males	37 (74%)
Number of females	13 (26%)
Mean Weight (kg)	69.97 ± 12.15
Mean Height (in meters)	1.63 ± 0.09
Mean BMI (kg/m^2)	26.41 ± 4.57

Table 1. Demographic details of patients enrolled in the study (n=50).

Parameter assessed	Mean value
Urine protein-creatinine ratio (gm/gm)	9.06 ± 5.38 gram/gram
Serum creatinine (mg/dl)	0.97 ± 0.42 mg/dl
Serum urea levels (mg/dl)	25.74 ± 13.01 mg/dl
Serum BUN (mg/dl)	12.03 ± 6.07 mg/dl
Serum albumin (gm/dl)	2.91 ± 0.73 mg/dl
eGFR (mL/min/1.73m^2)	90.43±34.58 mL/min/1.73m^2
Serum cholesterol(mg/dl)	275 ± 107.8 mg/dl

Table 2. Mean values of the measured biochemical parameters.

Association between Anti PLA2R and PLA2R tissue staining

Table 3a shows the distribution of serum anti PLA2R and PLA2R tissue staining in the study group. 43/50 (86%) patients were positive for tissue PLA2R staining and 21/50 patients (42%) were positive for serum Anti PLA2R.

Parameter	Serum PLA2R +ve	Serum PLA2R-ve	Total
Tissue PLA2 R +ve	20	23	43
Tissue PLA2R -ve	1	6	7
Total	21	29	

Table 3a. Association between Anti PLA2R and PLA2R tissue staining.

Serum anti-PLA2R titer in patients

Total of 21 (42%) patients had anti-PLA2R antibody above 20 RU/ml. Range was from 23.3 to 1279 RU/ml.

Comparison of groups having PLA2R positivity with PLA2R negativity

There was no significant association between various clinical, demographic and laboratory parameters among anti-PLA2R antibody positive and anti-PLA2R negative patients.

Correlation between serum anti PLA2R titre and biochemical variables

Serum creatinine had relevant statistical significance with moderate association with anti PLA2R titres ($p < 0.001$, $r = 0.485$). Detailed analysis of other biochemical parameters and their correlation with anti-PLA2R levels is displayed in Table 3b.

	R value (Correlation coefficient)	Interpretation of correlation	P value
Urine protein-creatinine ratio (gm/gm)	0.228	Positive	0.111
Serum creatinine (mg/dl)	0.485*	strong positive	0.0001*
Serum urea levels (mg/dl)	0.073	Positive	0.614
Serum albumin (gm/dl)	-0.260	Negative	0.068
eGFR (mL/min/1.73m^2)	-0.264	Negative	0.064

Table 3b. Correlation between serum anti PLA2R titer and biochemical parameters.

Association between serum anti-PLA2R titre and tissue PLA2R staining

Correlation between serum PLA2R antibody titre and PLA2R staining was done by ANOVA. It was observed that with increase in intensity of PLA2R staining on the tissue, the antibody titres increased but not significantly (p = 0.61).

Correlation between tissue PLA2R staining and biochemical parameters

Correlation between tissue PLA2R staining and various biochemical parameters were calculated by ANOVA (Table 4). Moderate positive correlation between urine protein-creatinine levels and tissue PLA2R staining grades and inverse correlation between albumin in serum and tissue PLA2R staining grades was observed on analysis.

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum	p-value
						Lower Bound	Upper Bound			
Urine PCR Baseline	Negative	3	6.4433	8.72677	5.0384	-15.2352	28.1218	1.36	16.52	0.040*
	2+	4	8.9175	6.68096	3.3404	-1.7134	19.5484	2.90	17.27	
	3+	14	6.0779	3.87404	1.0353	3.8411	8.3147	1.53	13.52	
	4+	29	10.7921	5.02340	0.9328	8.8813	12.7029	3.30	21.72	
	Total	50	9.0612	5.38331	0.7613	7.5313	10.5911	1.36	21.72	
Sr. Creatinine Baseline	Negative	3	0.7333	0.20817	0.1201	0.2162	1.2504	0.50	0.90	0.482
	2+	4	1.2075	0.29022	0.1451	0.7457	1.6693	0.80	1.43	
	3+	14	1.0150	0.49609	0.1325	0.7286	1.3014	0.50	2.50	
	4+	29	0.9379	0.41263	0.0766	0.7810	1.0949	0.40	2.20	
	Total	50	0.9688	0.42277	0.0597	0.8487	1.0889	0.40	2.50	
Urea Baseline	Negative	3	19.33	2.517	1.453	13.08	25.58	17	22	0.746
	2+	4	28.50	12.261	6.131	8.99	48.01	13	41	
	3+	14	27.64	13.602	3.635	19.79	35.50	12	62	
	4+	29	25.10	13.629	2.531	19.92	30.29	13	68	
	Total	50	25.74	13.004	1.839	22.04	29.44	12	68	
Serum Albumin Baseline	Negative	3	3.567	1.4572	0.8413	-0.053	7.186	1.9	4.6	0.026*
	2+	4	2.400	0.7257	0.3629	1.245	3.555	1.6	3.3	
	3+	14	3.243	0.6869	0.1836	2.846	3.639	1.8	4.3	
	4+	29	2.745	0.5761	0.1070	2.526	2.964	1.6	4.0	
	Total	50	2.906	0.7274	0.1029	2.699	3.113	1.6	4.6	
eGFR Baseline	Negative	3	127.000	70.1731	40.514	-47.319	301.320	68.6	204.9	0.145
	2+	4	66.125	26.5073	13.253	23.947	108.304	52.0	105.9	
	3+	14	88.811	30.7676	8.2230	71.046	106.575	31.9	142.8	
	4+	29	90.785	31.5854	5.8653	78.771	102.800	30.1	163.7	
	Total	50	90.433	34.5856	4.8911	80.603	100.262	30.1	204.9	

Table 4. Tabular representation along with the correlation interpretation and the p-values.

Comparison of groups having positive and negative serum anti-PLA2R

On analysis of the lab parameters in study participants having anti-PLA2R positive and anti-PLA2R negative membranous nephropathy, serum albumin was significantly lower in the anti-PLA2R antibody positive group compared to the negative group.

Comparison of data in groups having positive and negative tissue PLA2R in membranous nephropathy

On comparison of the biochemical parameters in patients having tissue PLA2R positive and negative group, no statistically significant differences were noted (Table 5).

Parameter assessed	Tissue PLA2R staining +ve (n=43)	Tissue PLA2R staining -ve (n=7)	p-value
Age (years)	46.2 ± 10.6	44.5 + 11.2	0.711
Urine Protein creatinine ratio (gm/gm)	9.2 ± 5.1	7.8 ± 7.0	0.529
Sr creatinine (mg/dl)	0.96 ± 0.43	1.0 ± 0.34	0.814
Urea (mg/dl)	25.9 ± 13.5	24.5 ± 10.1	0.801
Sr Albumin (gm/dl)	2.9 ± 0.6	2.9 ± 1.1	0.982
eGFR (mL/min/1.73m^2)	90.1 ± 30.9	92.2± 55.2	0.885
Duration of proteinuria (months)	9.0 ± 18.4	8.6 ± 12.4	0.948

***p<0.05 considered significant by unpaired t test**
****+ve = Positive, -ve = negative**
Table 5. Baseline parameters in patients having Tissue PLA2R positive and negative stained membranous nephropathy.

Discussion

In our research, the frequency of serum anti-PLA2R and glomerular PLA2R deposits in patients with MN and the correlation of both parameters with clinical implications were assessed which contributed to the novelty of this study.

There were patients with age varying from 17 to 67 years in this study. 34 (68%) patients were aged 40 or above. The lowest age of patient with MN in India is 5 years as reported by Kumar et al. [10]. The gender distribution (male: female) of patients with MN in this study was nearly 1:3. In our study, 12 (24%) patients were diabetic and 29 (58%) were hypertensive. In a study by Bhadauria et al. [11], 16 (11.9%) out of 134 diabetic patients had MN. 12 patients had features of only MN whereas the remaining 4 patients had combined features of both MN and diabetic nephropathy (DN) in the above-described study [11]. There were 29 (58%) patients with hypertension. In studies done by Gopalakrishnan et al. [8] and Roy et al. [12], the patients with hypertension presenting with MN were 16.6% and 24.7% respectively. Twelve (20.8%) of the 29 patients had arteriosclerosis histologically and among the 10 (20%) patients with diabetes and hypertension, 7 (70%) had arteriosclerosis in this study.

In a previous seminal study [8], patients with antibody positivity had severe degree of proteinuria compared to those without detectable antibody levels (urine PCR 5.98 g/g versus. 4.3 g/g, p = 0.0006). The albumin level in the serum was significantly lower in patients with antibody positivity (3.04 g/dl versus 3.5 g/dl, p = 0.0001). In our study, patients with antibody positivity had higher degree of proteinuria (urine PCR 10.1 ± 5.1 versus 8.3 ± 5.5; p = 0.253), lower albumin levels in serum (2.6 ± 0.6 versus 3.1 ± 0.7; p = 0.014). 43 (86%) had nephrotic range proteinuria with 23 (53.4%) being antibody positive. A total of 13 (26%) patients had nephrotic syndrome and these patients had a higher anti-PLA2R level in serum (123 versus 99 RU/ml) in this study.

Small studies done by Roy et al. [12], Ramachandran et al. [13], Gudipati A et al. [14], Rath et al. [15], Kaga et al. [16] and Pang et al. [17] corroborated with the findings of a recent meta-analysis by Dai et al. [18] which evaluated the utility and significance of serum anti-PLA2R and histological PLA2R staining. It found that serum anti-PLA2R had an overall sensitivity and specificity of 0.68 (95% CI, 0.61-074) and 0.97 (95% CI, 0.85-1.00) respectively [18]. Our study had similar findings of increased sensitivity of tissue PLA2R in correlating with proteinuria in PMN with a sensitivity of 88%.

The reason for absence of serum anti-PLA2R in some PMN patients could be because of one of the following reasons: sink effect/early stage of MN, antibodies against other antigens like Thrombospondin type-1 domain-containing 7A (THSD7A), Neural epidermal growth factor like 1 protein (NELL-1) and inability to identify secondary causes.

In our study, on looking at the correlation between duration of symptoms and the antibody titre, there was a mild negative correlation but there was no significance ($r = -0.123$, $p = 0.396$). In previous studies [16, 17], they noticed that anti-PLA2R was negative in patients who had recent exposure to immunosuppressants. In our study, none of the patients had prior exposure to immunosuppressive agents.

In this study, the sensitivity of tissue PLA2R deposits was 88% which complements the earlier studies [13, 15] all of which showed sensitivity above 80%. A total of 20 (40%) had both the positive anti-PLA2R antibodies and glomerular tissue deposits. In the previous research [13, 16, 17], antibody titres were measured and glomerular staining was quantified and it was noted that 59.6%, 56.6% and 44.7% patients were positive for both respectively. Reasons for this discordance might be due to false positivity of antibodies or not enough exposure of the antigen's epitope for the staining.

Association between anti-PLA2R titre and glomerular PLA2R staining intensity

Correlation between the antibody titre and tissue staining intensity was looked into in the studies mentioned above [13]. It was found that increased level of serum anti-PLA2R antibody translated to increased intensity of glomerular PLA2R staining although the correlation coefficient wasn't significant [13]. In our study, the titre and the tissue staining were correlated with ANOVA and it was noticed that as the intensity of the tissue staining increased, the titre of the anti-PLA2R antibody also increased, but was not significant ($p = 0.616$).

Relation between PLA2R status and various biochemical variables

In a previous study [8], proteinuria of nephrotic range was present in majority of patients with positive anti-PLA2R antibodies (IIF). Proteinuria ($p = 0.001$) and hypoalbuminemia ($p = 0.006$) was severe in patients with antibody positivity. Proteinuria had positive correlation with antibody intensity ($r = 0.465$, $p = 0.02$) in the above study [8]. Ramachandran et al. [13] found no association between the anti-PLA2R titre, proteinuria or albumin levels in serum. We found no differences in the baseline lab parameters with the anti PLA2R status in our study except serum albumin levels which had an inverse moderate association with anti-PLA2R antibody titres ($r = -0.468$, $p = 0.043$).

In an older study [17], anti-PLA2R antibody levels in serum was an efficient marker to indicate severity of PMN than glomerular PLA2R staining in contrast to our study which demonstrated both anti-PLA2R antibody titre as well as tissue PLA2R staining reflected and corelated well with the disease activity. The uniqueness of our study lies in the fact that there was only 42% positivity for serum anti-PLA2R in our study, contrasting with the 70-80% sensitivity of this marker in previous seminal studies [5, 6]. The probable scientific postulates for this low sensitivity of serum anti-PLA2R in our study group maybe clinical remission at the time of renal biopsy, presence of the antibodies against novel antigens responsible for PMN, very low titres of anti PLA2R at assessment, masking of antigenic epitopes by bound antibodies or sink phenomenon [18]. Our study challenges the diagnostic sensitivity of serum anti-PLA2R as a primary investigation tool in the algorithm of management of PMN. Future large-scale trials are required to incorporate a compendium of serological markers for diagnosis of PMN.

Conclusion

Serum anti-PLA2R wasn't a sensitive marker of primary membranous nephropathy in our study group emphasising the need to consider a compendium of serological markers for diagnosis of primary membranous nephropathy and to rely more on glomerular deposition of PLA2R as a better clinical indicator for PMN.

Future large-scale studies are required to identify a set of novel serological markers to reliably diagnose PMN with better sensitivity and specificity.

Limitations of the study

Small sample.

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