Research Article

Correlation of Anti-Phospholipase A2 Receptor Antibody Level with Disease Activity in Patients with Idiopathic Membranous Nephropathy

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Abstract

Background: Anti phospholipase A2 receptor (PLA2R) antibody detection in serum is a non-invasive, cost effective diagnostic test for idiopathic membranous nephropathy (iMN) precluding the need for a renal biopsy. Extensive research has demonstrated a strong correlation between serum anti-PLA2R antibodies and disease activity but no prior study has been conducted in Pakistan on this important aspect of iMN.

Objective: To ascertain the relationship between anti-PLA2R antibodies and disease activity in patients with biopsy proven iMN.

Methods: This cross sectional study was conducted at Nephrology Department, Mayo Hospital, Lahore from February to October, 2023. Clinical data of individuals with nephrotic syndrome, age >14 years were obtained upon enrollment in the study. Renal biopsy of patients was done and specimen sent for histopathology. The study encompassed eighty-three patients diagnosed with iMN, while the remaining individuals were excluded. Serum levels of anti-PLA2R antibodies of 83 patients with iMN were assessed.

Results: Out of 83 patients, 51 (61%) tested positive for anti-PLA2R antibody, while 32 (39%) were negative. The mean age of patients was 31.80 ±11.71 years. A significant positive relationship was identified between anti-PLA2R level and 24hour urinary protein ($r=0.535$, $p<0.001$), Low density lipoprotein ($r=0.357$, $p<0.05$). A significant negative relationship was found between anti-PLA2R level and albumin level ($r= -0.440$, $p<0.001$).

Conclusion: Anti-PLA2R antibody levels showed strong correlation with the clinical parameters reflecting the disease activity in iMN. In iMN, the sensitivity of anti-PLA2R antibody was determined to be moderate.

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Keywords | Anti-PLA2R antibodies, Idiopathic Membranous Nephropathy, Proteinuria.

Introduction

Membranous nephropathy (MN) is one of the most prevalent causes of nephrotic syndrome, particularly in the non-diabetic Caucasian population over the age of 40 years, with a projected incidence of 8-10 cases per 1 million people globally.[1] In Pakistan, MN ranks as the second most common cause of nephrotic syndrome ranging from 23.5%-36%.[2,3] The majority of patients exhibit significant proteinuria (>3.5 g/day), accompanied by peripheral edema, frothy urine and the potential for thromboembolic complications.[4]

In patients with MN, 75%–80% of cases are

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categorized as idiopathic MN (iMN) when the cause cannot be identified. Around 20-25% of cases are secondary to other underlying conditions like infections (hepatitis B, C), malignancy, drug reactions (NSAIDs, penicillamine, gold) and autoimmune diseases (systemic lupus erythematosus).[1,5] This differentiation is essential because the clinical management varies for each category. Evidence from the experimental models showed that glomerular immune deposits played a pivotal role in the pathogenesis of MN.[1,6] In their seminal study in 2009, Beck et al discovered the M-type transmembrane phospholipase A2 receptor (PLA2R), situated on glomerular podocytes, as the specific antigen in about 70% of the patients with iMN.[6] Similar results have been documented in multiple researches worldwide with sensitivity of above mentioned antibodies ranging from 60%-80% in iMN.[7,8,9,10]

Anti-PLA2R antibody detection in serum is a non-invasive, cost effective diagnostic test for iMN. According to data, a positive anti-PLA2R antibody by either ELISA or immunofluorescence assay has a high sensitivity (≈95%) for diagnosing iMN, possibly precluding the need for a renal biopsy and avoiding its complications.[11] Internationally extensive research has demonstrated a positive relationship between these antibodies and clinical status of disease. Furthermore, they prove valuable in predicting the disease trajectory and assessing a patient's response to treatment, making them a reliable biomarker for disease activity. However, there is no local data available regarding the level of anti-PLA2R and its correlation with disease activity. So, the aim of the study was to assess the correlation between the aforementioned antibodies and disease activity in patients with biopsy proven iMN.

Methods

This cross-sectional study took place at the Department of Renal Medicine at Mayo Hospital, Lahore, spanning from February to October 2023. Ethical approval was acquired from the Institutional Review Board of King Edward Medical University, Lahore (523 RC/KEMU, dated 22nd May 2022). Sample size of 74 patients was calculated by using 95% confidence level, 10% absolute precision with expected percentage of anti-PLA2R antibodies in iMN of 74%.[7] Written consent with full understanding was obtained from all participants with age more than 14 years presenting with nephrotic syndrome at nephrology outpatient department. Demographic data and clinical history with physical examination was taken upon enrollment in the study. To minimize the impact of confounders, we carefully evaluated each patient's medical history, including prior use of immunosuppressive therapies and other relevant treatments. Patients who had taken immunosuppressive drugs prior to the study were excluded to limit confounding effects, enhancing the reliability of the observed correlations. Baseline laboratory parameters of all patients including renal function tests (RFTs), lipid profile (fasting), twenty four hour urinary protein (24hUP) and serum albumin (Alb) were documented at the initiation of the study. All patients underwent (a diagnostic evaluation) to identify any potential secondary cause of MN. This comprehensive assessment incorporated the evaluation of viral markers (HBs Ag, Anti-HCV, Anti-HIV), ANA, anti-dsDNA, C3, C4, and chest X-ray, breast examination in women, prostate size/PSA in men and stool for occult blood. Renal biopsy of all the patients was done at Nephrology department, Mayo Hospital, Lahore and histopathology samples were sent for light microscopic and immunofluorescence testing. When renal biopsy reports were reviewed, out of the 92 patients diagnosed with MN, eighty three patients were enrolled in the study with a specific diagnosis of iMN on the basis of laboratory features and histologic characteristics while nine patients were eliminated due to secondary MN.

Serum level of anti-PLA2R antibodies in patients with iMN were assessed using readily available ELISA assays (EUROIMMUN AG). PLA2R coated Microplates were reacted with human serum, which were diluted 1:100 in buffer, and incubated for a duration of 30 minutes. After the incubation period, the antibodies were visualized by treating them with an anti-human-IgG horse-radish peroxidase conjugate for an additional 30 minutes. Using a microplate absorbance reader the optical density was quantified at 450 nm. In accordance with the manufacturer's instructions, values of 20 RU/mL or above were defined as positive, while levels below 20

RU/mL were deemed negative.

Statistical software IBM-SPSS v-23 was used to analyze the data. Age was described as Mean ± SD, whereas categorical data were expressed in frequencies. Correlation coefficient was employed to find the relationship between continuous variables, any association was investigated using chi-square test between categorical variables. Scatter plot was used to display continuous data. A p-value less than 0.05 was recognized as statistical significance.

Results

The total patients were 83, out of which anti-PLA2R positive were 51 (61%) and anti-PLAR negative were 32 (39%). The mean age of patients was 31.80 ± 11.71 years with range of 14 to 60 years. The mean duration of illness was 8.56± 7.98 years, 7.6% patients had diabetes, 40.2% had hypertension and 1 patient had a history of ischemic heart disease. A significant positive association was found between anti-PLA2R level and 24hUP (r=0.535, p<0.001), LDL (r=0.357, p<0.05) (Figure1, Figure 2 and Table No.1). A significant negative correlation (Figure 3) was found between anti PLA2R level and albumin level (r=-0.440, p<0.001). There was no significant correlation found between anti PLA2R and HDL, total cholesterol level, serum creatinine, serum urea and age (p>0.05).

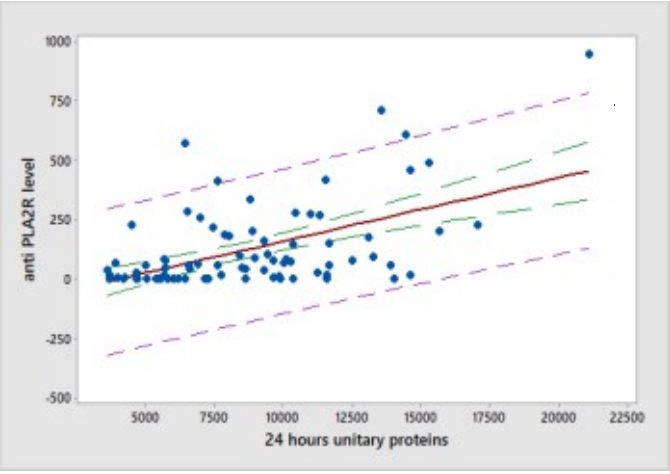

Figure-1: *Scatter plot illustrating the relationship between anti PLA2R and 24-hour urinary proteins*

Table 1: *Comparison of patient demographic and disease characteristics with anti-PLA2R detection*

Variable	Categories	Anti PLA2R		P-value
		Positive (N=51)	Negative (N=32)	
Gender	Male	37(72.5%)	18(36.3%)	0.126
	Female	14(27.5%)	14(43.7%)	
Serum creatinine (mg/dl)	<1.2	39(76.5%)	21(65.6%)	0.283
	≥ 1.2	12(23.5%)	11(34.4%)	
24 hours urinary proteins	low risk :3500-4000	5(9.8%)	3(9.4%)	0.026
(mg/24 hours)	moderate risk :4000-8000	14(27.5%)	18(56.3%)	
	high risk :>8000	32(62.7%)	11(34.3%)	
LDL levels	<130	1(2%)	3(9.3%)	0.125
(mg/dl)	≥130	50(98%)	29(90.7%)	
Total cholesterol	<200	1(2%)	1(3.1%)	0.736
(mg/dl)	≥ 200	50(98%)	31(96.9%)	
Serum urea levels	<40	30(58.8%)	19(59.4%)	0.960
(mg/dl)	≥ 40	21(41.2%)	13(40.6%)	
Serum albumin levels	<2.5	48(94%)	14(43.8%)	<0.001
(g/dl)	≥ 2.5	3 (6%)	18(56.2%)	
HDL levels	<60	47(92.2%)	31(96.9%)	0.379
(mg/dl)	≥ 60	4(7.8%)	1(3.1%)	

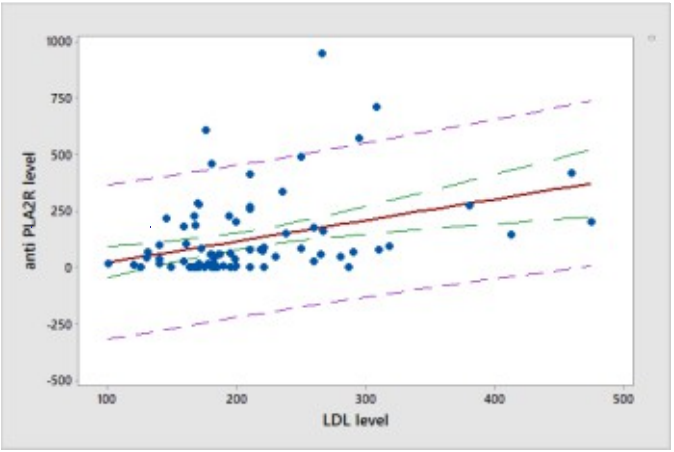

Figure-2: *Scatter plot illustrating the relationship between anti PLA2R and LDL Levels*

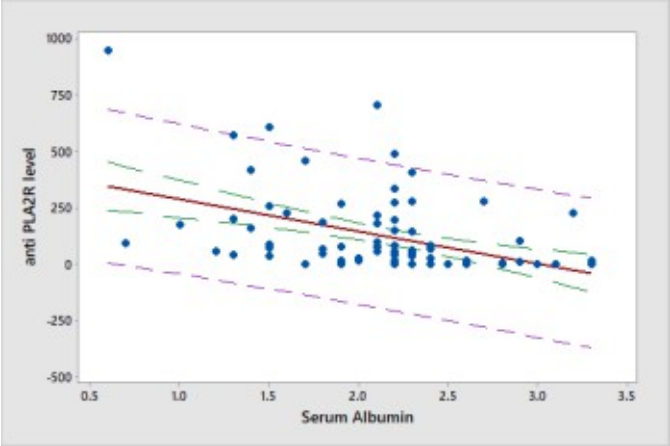

Figure-3: *Scatter plot illustrating the relationship between anti PLA2R and serum albumin.*

Discussion

Identification of anti-PLA2R antibodies in iMN has brought revolutionary change in diagnosis of MN. Within the scope of this study, the frequency of anti-PLA2R antibody positivity was 61.4% which was comparable to previous local studies and some international studies conducted in Asian countries like India and Japan.[12,13,14] However numerous researchers from western countries documented a high sensitivity rate of anti-PLA2R antibodies in iMN ranging between 70-80%.[7,8] The variations in the positivity rate of anti PLA2R antibodies in western literature and this study could be due to various reasons especially genetics and environment. iMN is considered as autoimmune disease which has heritable element of MN with strong association to the human leukocyte antigen (HLA) locus on chromosome 6. iMN is linked to specific HLA class II phenotypes such as HLA DRB1 and DRB3 in Asian population whereas HLA DQA1 is common in the White population.[15] A latest international genome-wide association study has established a genetic interaction between the PLA2R locus and class II HLA genes.[16] It is therefore postulated that genetic predisposition at the HLA class II locus can augment or diminish the display of immunogenic peptides originating from PLA2R to the immune system and have impact on sensitivity of anti PLA2R antibodies among different populations.

In addition to anti-PLA2R antibodies, alternative antibodies like Anti-Thrombospondin Type 1 domain containing 7A (THSD7A), present in 3-5% of iMN cases,[17] and Anti-neural epidermal growth factor like 1 protein (NELL-1) , found in 5-10% of patients, offer promising diagnostic value for anti-PLA2R-negative patients.[18] These antibodies found in individuals with iMN who tested negative for anti-PLA2R antibodies, are highly specific for primary MN with studies in secondary MN mostly yielding negative results. Integrating these alternative markers into diagnostic protocols could improve the sensitivity of non-invasive testing for iMN and reduce reliance on biopsies.

Moreover, assessing PLA2R antigen deposits in renal biopsy samples may enhance diagnostic accuracy, as some patients who tested negative for serum anti-PLA2R antibodies still exhibit glomerular PLA2R antigen deposits.[19,20] Li at al compared the presence of PLA2R antibody and glomerular deposits of PLA2R antigen in patients with iMN and found that 11 out of 17 patients who tested negative for aforementioned antibodies showed glomerular deposits of PLA2R antigen.[19] Similarly Chaitanya and colleagues showed that out of 50 patients, 42% were positive for these antibodies, whereas glomerular PLA2R deposition was observed in 86% of patients.[20]

The clinical utility of anti-PLA2R antibody testing extends beyond diagnosis, offering insights into disease activity. Higher anti-PLA2R antibody levels correlate with increased proteinuria and podocyte destruction,[21] as confirmed by our study, where a significant positive association was found between antibody levels and 24hUP.[7,8] The inverse relationship between serum albumin and anti-PLA2R antibody levels further supports the utility of these antibodies as indicators of disease severity.[22,23]

Dyslipidemias are also a usual manifestation of MN, with increased levels of LDL, VLDL, cholesterol and triglycerides and low levels of HDL.[4] Dyslipidemia accelerates the progression of atherosclerosis and are recognized as a significant contributor for cardiovascular disease in patients with nephrotic syndrome[4] Dong et al described that dyslipidemias especially hypercholesterolemia correlates with the degree of proteinuria and damage to renal tubules in individuals with iMN.[21] In current study, LDL showed a positive association with anti-PLA2R antibody

levels indicating that individuals with elevated antibody titers also demonstrated increased levels of LDL. Bihua Wang also found that LDL levels were notably elevated in patients with high anti-PLA2R levels thus supporting our finding.[22,23] Thus these findings imply that anti-PLA2R antibody is reliable indicator of disease activity of iMN.

Our study confirms the results of prior research regarding the correlation of anti PLA2R antibodies in patients with iMN in local population. It's worth noting that the detection of these antibodies shows several benefits, including being a fast, straightforward, sensitive, cost effective and non-invasive method for supporting the diagnosis of iMN precluding the requirement of renal biopsy. Quantification of anti-PLA2R antibody is not only useful for diagnosis and clinical correlation but it also carries prognostic value. Provatopoulou et al showed that antibody levels strongly predicted achievement of complete remission of nephrotic syndrome at the end of treatment.[14] Furthermore, among patients with detectable antibody level, high baseline titers were found to be asociated with worse long-term outcomes and increased risk to develop end stage kidney disease.[9,14] In clinical practice, monitoring anti-PLA2R antibody levels could guide treatment decisions, particularly in tailoring immuno-suppressive therapy and evaluating response to treatment.

Our study had some limitations. Our sample size was comparatively small. Our study was limited to a single center and lacked a control group. There is a need for follow-up studies with inclusion of a control group to reinforce the conclusions about the specificity of anti-PLA2R in diagnosing iMN. Within the scope of this study, we have not performed PLA2R antigen glomerular staining in renal biopsy samples, but in future studies should be done to establish the correlation between serum anti-PLA2R antibodies and PLA2R antigen deposits in glomeruli of patients with iMN in local population.

Conclusion

Anti-PLA2R antibody levels showed strong correlation with the clinical parameters reflecting the disease activity in iMN. The sensitivity of anti-PLA2R antibody was determined to be moderate in patients with iMN.

Thus, anti-PLA2R positivity by ELISA could potentially replace kidney biopsy in patients with suspected MN, and therefore should be a part of screening panel for nephrotic syndrome.

Ethical Approval: The Institutional Review Board, King Edward Medial University, Lahore, Pakistan approved this study vide letter No.523/ RC/ KEMU.

Conflict of Interest: The authors declare no conflict of interest.

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Author Contribution

FQ: Conception & design, acquisition of data, analysis & interpretation of data, drafting of article.

MA: Critical revision for important intellectual content, final approval.

MSP: Acquisition of data, analysis & interpretation of data.

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