Anti-Phospholipase A2 Receptor Antibodies in Biopsy-Proven Idiopathic Membranous Nephropathy: A Report from the Pakistani Population

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ABSTRACT

This study aimed to determine the percentage of anti-phospholipase A2 receptor (PLA2R) autoantibody positivity in a cross-section of 74 Pakistani patients with biopsy-proven idiopathic membranous nephropathy. It was an observational study conducted from December 2018 to June 2019. Seventy-four (n=74) consecutive biopsy-proven cases of membranous nephropathy, aged between 12-60 years, were included. Out of them, 63.5% (n=47) were males and 36.5% (n=27) were females. The mean age was 40.2±16.7 years. Anti-PLA2R antibodies were positive in 35 (47.3%) patients, negative in 38 (51.4%) patients, and borderline positive in 1(1.35%) patient. A significant difference (p ≤ 0.05) was noted among different levels of antibodies in both genders. Anti-PLA2R antibodies were detected in approximately half of the diagnosed idiopathic membranous nephropathy cases. However, this percentage was much lower than the studies conducted on other populations around the globe, indicating a possible pathogenic role of other antigens in our population.

Key Words: Membranous nephropathy, Phospholipase A2 receptors, Renal biopsy.


Membranous nephropathy is the most common cause of adult-onset nephrotic syndrome, which includes idiopathic or primary and secondary membranous nephropathy. Primary membranous nephropathy accounts for 80-85% of cases and secondary occurs in 20% of the cases. It is an immunologically mediated disorder, which is caused by sub-epithelial deposition of immune complexes beneath the podocytic membrane. Phospholipase A2 receptor is the transmembrane protein located in the podocytic membrane. These (anti-PLA2R) antibodies are non-complement IgG-4 subclass of antibodies.

The rationale of this study was to find out the predominance of anti PLA2R receptor antibodies, detected by the ELISA method, in biopsy-proven patients of primary membranous nephropathy. This study has not been conducted anywhere else in Pakistan before. This non-invasive test can be utilized for diagnostic and monitoring purposes as well as in the future management of our membranous nephropathy patients.

A cross-sectional survey was conducted from December 2018 to June 2019 in the Department of Nephrology at Shifa International Hospital, Islamabad, after approval from the Ethical Committee (Approval number: 158-648-2019). The sampling technique was consecutive sampling from the outpatient department (OPD). Using WHO sample size calculator, with the confidence interval of 95%, predictable population percentage of 74%, absolute precision of 10%, the sample size calculated was 74 participants, which were included in this research. Patients between 12 to 60 years of age were included. These patients had light microscopic evidence of sub-epithelial immune complex deposits, along with glomerular basement membrane thickening and immunofluorescence microscopic evidence of IgG and c3 dominant immune deposits. Patients below 12 and above 60 years, and all diagnosed cases of secondary membranous nephropathy, were excluded.

Age, gender, level of proteinuria, serum albumin, serum total cholesterol levels, and serum creatinine at the time of renal biopsy were noted. All patients with biopsy-proven membranous nephropathy underwent testing for anti-PLAR2 antibodies by ELISA method, which was verified by the hospital microbiologist. Antibody levels above 20 RU/mL were considered as positive, between 14 RU/mL to 20 RU/mL as borderline, and below 14 RU/mL as negative.

The collected data was analysed with SPSS version 23. The categorical variables like gender, presence or absence of anti-PLA2R antibodies were represented as proportion and frequency.
The normality of distributions of continuous variables was checked by K-S test. The quantitative variables like level of proteinuria, serum albumin, total cholesterol, and serum creatinine were expressed using median and interquartile range (IQR). Age was expressed using mean and standard deviation. Anti-PLA2R antibodies were stratified to control effect modifiers like age and gender. Post-stratification Chi-square test was applied. The p-value of ≤0.05 was considered statistically significant.

Out of total seventy-four allocated patients, 27 (36.5%) were females and 47(63.5%) were males. The mean age of the patients was 40.2 years ±16.7 SD. Results of laboratory investigations of these patients include median serum creatinine of 0.955 mg/dL (IQR: 0.715-1.610mg/dL), median proteinuria of 5.223 g/24 hours (IQR: 3.560-8.803g/24 hours), median serum albumin of 2.545 g/dL (IQR: 2.085-3.100 g/dL) and median serum cholesterol level of 277.5 mg/dL (IQR: 218.5- 356.25 mg/dL). Out of the 74 studied patients, anti-PLA2R autoantibodies were positive in 47.3% (n=35), negative in 51.4% (n=38) and borderline in 1.4% (n=1) patients. Anti-PLA2R levels were stratified for demographic effect modifiers like gender and age groups. A statistically significant difference (p=0.011) was noted in gender, while no significant difference (p=0.449) was present among various age groups (Table I).

Several studies have been published regarding anti-PLA2R prevalence in the Asian population. In this study, 47.3% patients had anti-PLA2R positivity, which was significantly low compared to other Asian populations excluding Japan. There are different techniques for anti-PLA2R antibodies identification, which include Western blot, indirect immunofluorescence, and ELISA. The diagnostic efficacy of these methods has been studied in different studies. Western blotting has a complicated protocol and indirect immunofluorescence has the limitation that it is a semi-quantitative analysis. On the other hand, the ELISA technique has the advantage of being quantitative, more objective, and simpler technique.

Although the reported prevalence of anti-PLA2R antibodies is around 70-80% in primary membranous nephropathy, yet ethical difference has been suggested in their prevalence. The low positive percentage of these auto-antibodies in idio-pathic membranous nephropathy in our population as compared to most other Asian populations could possibly be due to the effects of immunosuppression, possible significant role of other antibodies in the pathogenesis of membranous nephropathy in our population like anti-thrombospondin type 1 domain containing 7A antibodies and use of diagnostic technique different from techniques studied in Chinese, Indian, Iranian and Korean population groups.

The detected positivity of these autoantibodies in our study population is much closer to the Japanese and Greek study groups. Over the past decade, several different antigens have been found to be linked to the pathogenesis of membranous nephropathy, and the detection of antibodies to these antigens have diagnostic significance. The antigens include phospholipase A2, thrombospondin domain 7A and newly found two antigens; NELL-1 and exostosin.

Besides this, in one Japanese study, the prevalence of anti-enolase antibodies was reported to be 70% in both primary and secondary membranous nephropathy. These findings thus indicate that there might be a significant role of some other antibodies in the pathogenesis of disease in this study population.

In this study, PLA2R positivity has been seen in 80% of male patients and 20% of females, indicating a positive association of anti-PLA2R positivity in the male gender. This finding has been supported by another recently published study, in which 75.3% of the males have anti-PLA2R positivity.

The significance of the detection of these autoantibodies has been highlighted in several studies. Detection of these antibodies aids in the diagnosis, prognosis, monitoring the disease activity, response to therapy, differentiating primary from secondary membranous nephropathy, and predicting the possibility of post-transplant recurrence of membranous nephropathy. In addition to this, recently it has been proposed that in patients with nephrotic syndrome, normal renal function, and after ruling out secondary membranous nephropathy, the detection of serum anti-PLA2R antibodies obviates the need of performing the renal biopsy, a more invasive diagnostic modality.

### Table I: Gender and age-wise stratification of Anti-PLA2R antibodies.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Negative</th>
<th>Borderline</th>
<th>Positive</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (47.4%)</td>
<td>1 (100%)</td>
<td>28 (80.0%)</td>
<td>47 (63.5%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Female</td>
<td>20 (52.6%)</td>
<td>0</td>
<td>7 (20.0%)</td>
<td>27 (36.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38 (100%)</td>
<td>1 (100%)</td>
<td>35 (100%)</td>
<td>74 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group</th>
<th>Negative</th>
<th>Borderline</th>
<th>Positive</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤20 years</td>
<td>7 (18.4%)</td>
<td>0</td>
<td>3 (8.6%)</td>
<td>10 (13.5%)</td>
<td>0.449</td>
</tr>
<tr>
<td>20-40 years</td>
<td>18 (47.4%)</td>
<td>0</td>
<td>15 (42.9%)</td>
<td>33 (44.6%)</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>13 (34.2%)</td>
<td>1 (100%)</td>
<td>17 (48.6%)</td>
<td>31 (41.9%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>38 (100%)</td>
<td>1 (100%)</td>
<td>35 (100%)</td>
<td>74 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

*p-values are calculated via pearson Chi-square test.*
This study has several limitations, which include small sample size and not taking into consideration the effects of immunosuppression on antibodies level. The findings of this study are needed to be validated on the large sample size.

This study showed that anti-PLA2R antibodies were positive in about half of the total study population with biopsy-proven membranous nephropathy. As indicated above, measuring these antibody titers is highly specific for the detection of idiopathic membranous nephropathy, therefore, this investigation must routinely be performed in patients with clinical presentation of nephrotic syndrome.

**DISCLOSURE:**
This is a dissertation-based article.

**COMPETING INTEREST:**
The authors declared no competing interest.

**AUTHORS’ CONTRIBUTION:**
NS: Contributed to study concept, design, intellectual content, literature search, data collection and analysis, and manuscript writing and review.
SK: Contributed to intellectual content, data collection and analysis, literature search, and manuscript writing.
SNM: Contributed to study design, concept, and literature search.
MH: Contributed to study design, literature search, and manuscript writing.
All authors approved the final version of the manuscript to be published.

**REFERENCES**


