Original Article

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An Evaluation of the Level of Anti-phospholipase A2 Receptor (PLA2R) Antibody in Different Pathological Types of Glomerulonephritis

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ABSTRACT

BACKGROUND AND OBJECTIVE: Nowadays, anti-phospholipase A2 receptor (PLA2R) antibody is recognized as a diagnostic biomarker for idiopathic membranous nephropathy (iMN) and is also used to assess disease progression. Considering the differences in the prevalence of this antibody in various geographic regions, this study aims to evaluate the level of anti-PLA2R antibody in different types of glomerulonephritis and its relationship with proteinuria and creatinine clearance.

METHODS: This cross-sectional study was conducted among 115 patients with diagnosis of glomerulonephritis and nephrotic syndrome. The patients were divided into three categories of iMN (34 patients), secondary membranous nephropathy (9 patients) and other types of glomerulonephritis (72 patients) based on their clinical history and test results. Demographic data, latest creatinine level, volume, protein and the 24-hour urine creatinine were recorded and the creatinine clearance was calculated. The serum level of anti-PLA2R antibody was measured and the level of antibody ≥ 20 RU/ml was considered positive. The level of antibody was compared between the three groups and the relationship between antibody levels and creatinine clearance and proteinuria was assessed in patients with iMN.

FINDINGS: The mean age of the patients was 38±13 years. 81 patients (70%) were female. The mean (SD) antibody level in iMN patients was significantly higher than secondary membranous nephropathy and other types of glomerulopathy (129.9±190.1, 2.6±1.7, 2.0±1.6, respectively, P<0.0001). The antibody level was positive in 47% of the patients with iMN, while it was not positive in any of the other types of glomerulopathy (P<0.001). However, there was no significant relationship between antibody levels and creatinine clearance or 24-hour proteinuria in patients with iMN.

CONCLUSION: Results of the present study demonstrated that the presence of anti-PLA2R antibody specifically indicates idiopathic membranous glomerulonephritis. However, antibody level is not associated with disease severity.

KEY WORDS: *Glomerulonephritis, Idiopathic membranous nephropathy, Anti-PLA2R antibody, Creatinine clearance, Proteinuria.*

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Introduction

Membranous nephropathy is one of the important causes of nephrotic syndrome (1) and its prevalence is 1.2 per 100 thousand people every year (2). This disease is diagnosed with sub-epithelial immune deposits, proteinuria mediated by complement and diffuse thickening of the glomerular capillary basement membrane (3 – 7). About 75 – 80% of cases are idiopathic, since no specific etiology is diagnosed for it. The remaining cases are classified as secondary type as they are associated with other diseases such as systemic lupus erythematosus, cancers, bacterial or viral infections, and drug toxicity (8, 9).

Hence, in order to improve clinical treatment and outcomes it is important to distinguish between idiopathic and secondary types of the disease (3, 10). Recent studies show that idiopathic membranous nephropathy (iMN) is an autoimmune disease (5 – 7, 11, 12). The M-type phospholipase A2 receptor (PLA2R) is the first discovered human autoantigen in adults, which is a member of mannose receptor family and is found in natural human glomeruli as a trans-membrane receptor (11).

M-type PLA2R is known as an important anti-cancer target in autoimmune iMN (13). Several studies indicated that about 70 – 80% of patients with iMN have anti-PLA2R antibodies (12). Anti-PLA2R antibodies found in serum samples of patients with iMN are categorized under IgG4 subclass (11). Vice versa, most patients with secondary membranous nephropathy are negative regarding this antibody (12). However, it is reported in one study that some patients with secondary MN due to hepatitis, cancer and lupus had anti-PLA2R antibodies (14).

Since anti-PLA2R antibody level is associated with the clinical activity of iMN, it can be used for monitoring response to treatment. Therefore, anti-PLA2R antibody has been suggested as a diagnostic biomarker for iMN (13,15,16). Compared with kidney biopsy, which is an invasive diagnostic method and may be accompanied by complications such as glomerular damage or other serious complications, serologic test for anti-PLA2R antibody is fairly simpler and safer than pathologic tests. Therefore, it can provide a quick diagnostic method for clinical researchers. The prevalence of this antibody has been reported to be 45 – 86% in patients with iMN in several studies (8, 11,14,17–19) and studies in this field have reported the sensitivity of this antibody to be 52 – 98.4% (11,14,17,20,21). Therefore, the efficiency of anti-PLA2R antibody as a diagnostic tool for iMN is still a controversy (22). On the hand, the prevalence of anti-PLA2R antibody may be diverse in different geographical regions (23). Considering that only one study has been conducted in Iran regarding anti-PLA2R antibody in patients with iMN (24), the present study was conducted to quantitatively and qualitatively analyze the anti-PLA2R antibody level in different pathological types of glomerulonephritis and its relationship with the level of creatinine clearance and proteinuria in Iran.

Methods

After getting permission from the ethics committee of Iran University of Medical Sciences (105.35.D.94) and obtaining informed written consent from patients, this cross-sectional study was conducted among 115 patients diagnosed with glomerulonephritis and nephrotic syndrome (proteinuria more than 3.5 at the time of diagnosis) who were admitted to nephrology clinic of Hashemi Nejad Hospital in Tehran. Glomerulonephritis was diagnosed based on patient's history, records and in some cases, kidney biopsy results. The patients were divided into three groups of iMN, secondary membranous nephropathy and other types of glomerulonephritis.

When no specific cause was found for membranous nephropathy based on clinical examination, hepatitis profile test, serology, S/E and finally kidney biopsy, the patient was considered to have iMN. The secondary cause for membranous nephropathy in this study was lupus nephritis stage V (25). The patients' information including demographic data (age, gender and weight), latest creatinine and type of glomerulonephritis were recorded. In addition, the data of the latest 24-hour urine test including volume, protein and creatinine were also recorded. The creatinine clearance was calculated based on Cockcroft – Gault formula.

Then, a blood sample was obtained from patients and was frozen until the time of serologic test. After samples were collected completely, serologic test was performed using indirect immunofluorescence test (IFA EUROIMMUN AG, Lubeck, Germany) (17) and the anti-PLA2R antibody level was determined in patients. The test results were categorized as follows: <14 RU/ml=negative, 14–20 RU/ml = borderline and ≥20 RU/ml=positive. Finally, anti-PLA2R antibody level was qualitatively and quantitatively compared

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between different groups of glomerulonephritis. The relationship between anti-PLA2R antibody level and creatinine, creatinine clearance and proteinuria was also analyzed in patients with iMN. SPSS ver.22 was used to analyze the obtained data. The qualitative variables were describes using frequency and percentage while the quantitative variables were describes using mean and standard deviation, median and interquartile range. In order to compare the anti-PLA2R antibody level between different groups of glomerulonephritis, Non-Parametric Mann-Whitney U Test was used for quantitative analysis and Chi-square test was used for qualitative analysis.

Furthermore, Spearman correlation coefficient was used to analyze the relationship between the anti-PLA2R antibody level and proteinuria and creatinine clearance in patients with iMN and Non-parametric Kruskal Wallis test was used to compared the anti-PLA2R antibody level in various levels of proteinuria. The $p<0.05$ was considered as significance level.

Results

Of 115 patients in this study, 81 patients (70%) were female and 34 patients (30%) were male. Their age range and mean age were 15–76 years and 38±13 years, respectively. The range and mean serum creatinine were 0.7–18.8 mg/dl and 1.8±2.1 mg/dl, respectively. The range and mean 24-hour urine proteinuria were respectively 299–34000 mg and 5699±5413 mg, while the range and mean creatinine clearance were 8–173 and 78±39 mL/min/1.73m^2, respectively. Regarding different types of glomerulonephritis, iMN was present in 34 patients (30%), secondary membranous glomerulopathy was present in 9 patients (7.5%) and other types of glomerulopathy were present in the rest of patients (62.5%). Secondary membranous glomerulopathy in this study only included lupus nephritis stage V and secondary cases were not observed in the causes of hepatitis B and C, cancer and other causes (table 1).

The median of the anti-PLA2R antibody level was 7.2 in patients with iMN, 2.4 in patients with secondary membranous nephropathy and 1.6 in other glomerulopathies, while it was significantly higher in patients with iMN ($p<0.001$) (table 2).

Table 1. Different types of glomerulonephritis in the studied patients

Glomerulonephritis	Frequency (%)
Idiopathic Membranous Nephropathy (iMN)	34(30)
Minimal change disease	20(17)
FSGS	14(12)
IgA Nephropathy	10(8.5)
Secondary MN (Lupus Nephritis)	9(7.5)
Lupus Nephritis (Others)	7(6)
Diabetic Nephropathy	4(3.5)
Renal Amyloidosis	4(3.5)
Focal Crescentic GN (Pauci immune)	3(2.5)
Postinfectious GN	3(2.5)
C3 Glumerulopathy	2(2)
Thrombotic Microangiopathy	2(2)
Diffuse Membrano-proliferative GN	2(2)
Diffuse sclerotic & Focal Crescentic GN (Pauci immune)	1(1)

The qualitative comparison of the anti-PLA2R antibody level demonstrated that the anti-PLA2R antibody was not positive in any of the patients with secondary membranous nephropathy and other types of glomerulonephritis, whereas 47% of patients with iMN had positive antibody, which was significantly higher ($p<0.001$) (table 3).

There was no statistically significant association between anti-PLA2R antibody level and any case of creatinine, creatinine clearance and 24-hour urine proteinuria. Although the median of anti-PLA2R antibody in patients with proteinuria higher than 6 g/day was higher than other groups, there was no significant difference between various levels of proteinuria (Table 4).

Table 2. Quantitative analysis of anti-PLA2R antibody level in the studied patients

Variable	Median (interquartile range)	Mean±SD	Range	Number	P-value
Idiopathic membranous nephropathy	7.2(1.3-265.9)	129.9±190.1	0.1-703.1	34	
Secondary membranous nephropathy	2.4(1.2-3.8)	2.6±1.7	0.6-5.9	9	<0.001
Other types of glomerulonephritis	1.6(1.0-2.7)	2.0±1.6	0.1-6.9	72	
All patients	1.9(1.1-3.7)	39.9±117.9	0.1-703.1	115	-
* Mann Whitney U test					

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Table 3. The status of anti-PLA2R antibody level in the studied patients

Variable	Positive (≥20 RU/ml) N(%)	Borderline (14 – 20 RU/ml) N(%)	Negative (<14 RU/ml) N(%)	P-value*
Idiopathic membranous nephropathy	16 (47)	0(0)	18(53)	
Secondary membranous nephropathy	0 (0)	0(0)	9(100)	<0.001
Other types of glomerulonephritis	0 (0)	0(0)	72(100)	
All patients	16 (14)	0(0)	99(86)	-
* Chi-square test				

Table 4. Comparison of anti-PLA2R antibody levels in 24-hour urine proteinuria levels in patients with idiopathic membranous nephropathy

24-hour urine proteinuria	Antibody median (interquartile range)	N(%)	P-value*
≤3.5 g/day	3.2(1.3-47.6)	9(27)	
3.5-6 g/day	2.7(1.9-92.1)	12(35)	0.208
>6 g/day	349.6(0.9-427.3)	13(38)	
*Kruskal Wallis test			

Discussion

Results of this study demonstrated that anti-PLA2R antibody level in patients with iMN is significantly higher. In terms of quality, none of the patients with membranous nephropathy and other types of glomerulonephritis had positive anti-PLA2R antibody, while 47% of patients with iMN had positive anti-PLA2R antibody, which was significantly higher. However, there was no significant association between anti-PLA2R antibody level and creatinine, creatinine clearance and 24-hour urine proteinuria.

At least four autoantigens have been found in patients with membranous nephropathy so far. One of them is phospholipase A2 receptor, which is naturally expressed in podocytes (24, 26). In fact, PLA2R is a transmembrane glycoprotein type I, which is a member of the C-type family of mammalian lectins such as mannose receptors. PLA2R regulates some biologic responses created by secretory phospholipase A2 (11, 27). It is suggested that group IB path in secretory phospholipase A2 and PLA2R has a critical role in the production of proinflammatory cytokines. It is also stated that PLA2R plays a role in the clearance of secretory phospholipase A2 (28).

Therefore, determining the anti-PLA2R antibody level in patients with nephrotic syndrome can help diagnose iMN and differentiating it from the secondary type (20, 27 and 28). Studies in recent years indicated that anti-PLA2R antibody is present in different types of iMN and is often not found in the secondary type, other types of glomerulonephritis or normal people (11, 15, 17, 20, 22–24, 29–32). However, Qin et al. reported positive anti-PLA2R antibody in some cases of membranes nephropathy caused by lupus, hepatitis and cancer (14). The prevalence of this antibody was stated to be 45 – 86% (8, 11, 14, 17–19). Our study also demonstrated that 47% of patients with iMN had positive anti-PLA2R antibody, while none of the patients with secondary membranous nephropathy or other types of glomerulonephritis had positive anti-PLA2R antibody. However, the prevalence of anti-PLA2R antibody in our study was lower than another study conducted by Ardalan et al. in Iran, which reported a prevalence of 74% (24).

This difference in the prevalence of anti-PLA2R antibody may be attributed to the method used to measure the antibody, the studied population, treatment status of patients and the geographical region (23). Although several studies have indicated the relationship between anti-PLA2R antibody level, the disease activity and proteinuria (20, 33–36). However, our study did not find any relationship between them. In this regard, our study is only consistent with another study by Ardalan et al. in Iran (24).

Considering that the prevalence of anti-PLA2R antibody may be different depending on the geographical region (23), its relationship with disease activity and level of proteinuria may also be different. In addition, the treatment status of patients and the use

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of immunosuppressive drugs may also cause such differences. Therefore, it is necessary to do such researches in any geographical region. Since our study was cross-sectional and the antibody level was only measured once, analyzing the effect of treatment on antibody level was not possible, which is one of the limitations of the present study.

Therefore, it is suggested that future studies in Iran be prospective studies that analyze the process of disease according to anti-PLA2R antibody level and the response to treatments and their viability. In summary, our study demonstrated that 47% of patients with iMN had positive anti-PLA2R antibody, while none of the patients with secondary membranous nephropathy or other types of glomerulonephritis had positive anti-PLA2R antibody. However, there was no statistically significant association between anti-PLA2R antibody level and any case of creatinine, creatinine clearance and 24-hour urine proteinuria.

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