ORIGINAL RESEARCH ARTICLE

The correlation between Serum phospholipase A2 receptor antibody and clinicopathological features in patients with membranous nephropathy

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ABSTRACT

Objective: To assess the correlation between Serum phospholipase A2 receptor antibody and clinicopathological features in patients with membranous nephropathy. **Method:** The patients being hospitalized for renal biopsy were selected in this study from January 2016 to January 2018. And normal controls were randomly selected; all the patients were divided into idiopathic membranous nephropathy and non-idiopathic membranous nephropathy groups; patients with idiopathic membranous nephropathy were divided into three groups, namely stage I, stage II and stage III; using software for statistical analysis. **Results:** A total of 357 patients were enrolled, including 155 patients with idiopathic membranous nephropathy, 183 patients with non-idiopathic membranous nephropathy, and 19 cases for normal controls. The average age of the idiopathic membranous nephropathy (IMN) group is higher than that of the membranous nephropathy group (P = 0.01). Different pathological stages of idiopathic membranous nephropathy general clinical characteristics analysis results showed that the age, cys c, serum creatinine (Scr) in stage III membranous nephropathy group were higher than those of the stage I and II membranous nephropathy (P values were 0.003, 0.000 and 0.000 respectively); titers of serum phospholipase A2 receptors antibody with stage II and III membranous nephropathy higher than the stage I membranous nephropathy group (P = 0.006); serum albumin (Alb) levels correlated inversely with serum anti-PLA2R antibody titers (rs = –0.234, P = 0.003), serum antiphospholipase A2 receptor (PLA2R) antibody titer level in patients with idiopathic membranous nephropathy was significantly higher than that in patients with non-membranous nephropathy (P < 0.001). **Conclusion:** Baseline titer of serum anti-PLA2R antibody is negatively correlated with Alb in the IMN patients，and serum anti-PLA2R antibody level in patients with stage I IMN was significantly lower than stage II and III IMN patients.

Keywords: Phospholipase A2 Receptor Antibody; Idiopathic Membranous Nephropathy; Clinicopathological

ARTICLE INFO

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1. Introduction

Membranous nephropathy is one of the common histopathological types in primary glomerular diseases. It is a group of diseases characterized by subepithelial immune complex deposition of glomerular basement membrane (GBM) with diffuse thickening of GBM[1]. According to the cause, it can be divided into: (1) secondary membranous nephropathy; (2) familial membranous kidney disease; (3) idiopathic membranous nephropathy (IMN). IMN accounts for about 70% of all membranous nephropathy. Because the etiology of IMN is unclear, although it has been recognized that IMN is due to the autoantibodies against some antigens on the glomerular epithelial cell membrane, which fall off and deposit under the epithelial cells after binding with

the antigen, reactivate the complement and form a membrane attack complex C5b-9, causing a series of renal injury, its exact pathogenesis is not clear[2].Therefore, the diagnosis, treatment and prognosis of IMN have been controversial for a long time. With the deepening of research, Beck *et al.*[3] found anti phospholipase A2 receptor (PLA2R) antibodies in the blood of most IMN patients through Western blotting and mass spectrometry in 2009, and inferred that a glycoprotein-m-type PLA2R with a molecular weight of 185 kD on podocytes of IMN patients is the main target antigen. However, no anti-PLA2R antibody was detected in the blood of non IMN patients, suggesting that anti-PLA2R antibody is a unique molecular biological change of IMN, which is of great significance for the diagnosis and differential diagnosis of IMN. Qin *et al.*[4] also verified the value of detecting serum anti-PLA2R antibody in IMN patients in Chinese population. Many studies have confirmed that serum PLA2R antibody is related to the disease activity of IMN, but the relationship between serum PLA2R antibody titer and clinical biochemical indexes such as Alb and proteinuria is still controversial. There are also differences in serum PLA2R antibody titer in patients with different pathological stages. The purpose of this study is to clarify the relationship between serum PLA2R antibody and serum protein to explore the relationship between quantitative proteinuria, cystatin c (cys c), blood lipid, creatinine and other clinical and biochemical indexes, and to explore the application value of PLA2R antibody in early detection and differentiation of pathological stages of membranous nephropathy.

2. Data and methods

2.1 Research objects

Patients who underwent renal biopsy in the Department of Nephrology of Ganzhou people's Hospital from January 2016 to January 2018 were selected. Clinical indexes such as age, gender, Alb, cys c, blood cholesterol (CHO), blood creatinine (Scr) and proteinuria were collected, and randomly selected health physical examiners. The serum PLA2R antibody of healthy subjects was detect-

ed by enzyme-linked immunosorbent assay (ELISA). According to the results of pathological light microscope, immunofluorescence and electron microscope of renal biopsy, they were divided into the IMN group and the non-membranous nephropathy group. IMN patients were divided into stage I, stage II and stage III groups according to the results of the pathological stage. All patients were not given glucocorticoid before renal biopsy and immunosuppressive therapy.

2.2 Detection of serum anti-PLA2R antibody

5 mL of blood was drawn from patients before renal biopsy, and the anti-PLA2R antibody titer in blood samples was quantitatively detected by enzyme-linked immunosorbent assay (ELISA). The anti-PLA2R antibody IgG detection kit (ELISA) was produced by the Germany company EURO-IMMUN, and was operated in strict accordance with the instructions. If the serum anti-PLA2R antibody titer was greater than 14 RU mL^{-1}, it was determined as positive.

2.3 Pathological diagnosis of renal biopsy

After excluding contraindications and signing the informed consent, all patients underwent right renal inferior pole puncture under ultrasound guidance, and the renal tissues were routinely examined by HE, PAS, PASM, Masson staining, light microscope, immunofluorescence and electron microscope. The Ehrenreich Chung staging method was used for the staging of membranous nephropathy[5].

2.4 Statistical method

SPSS 22.0 statistical software was used for analysis. The quantitative data were subject to normal distribution and expressed by mean ± standard deviation. One-way analysis of variance (ANOVA) was used for inter group comparison, and the SNK method was used for multiple comparison. If not obeying the normal distribution, it was expressed by the median M (Min, Max). The nonparametric test of multiple independent sample data was used for the comparison between groups. When it is statistically significant, the Nemenyi method was used for multiple comparison. Qualitative data were expressed by cases (%), and comparison between

groups was tested by χ^2. Spearman correlation coefficient was used to analyze the correlation between variables, and $P < 0.05$ was statistically significant.

3. Results

3.1 Basic data of enrolled patients

A total of 357 patients were selected, including 155 patients with IMN, 99 males and 56 females: the ratio of males to females was 1.77:1, and the average age was (50.16 ± 10.60) years. There were 183 cases of non-membranous nephropathy, including 116 males and 67 females: the ratio of males to females was 1.73:1, with an average age of (43.32 ± 16.41) years; it included 69 cases of IgA nephropathy, 26 cases of minimal change, 28 cases of focal segmental glomerulosclerosis (FSGS), 18 cases of podocyte disease (MCD or potential FSGS), 28 cases of mesangial proliferative glomerulonephritis, and 8 cases of diabetic nephropathy and 6 cases of Henoch Schonlein purpura nephritis. There were 19 healthy cases as the control group. There was no significant difference in gender ratio between IMN group and non-membranous nephropathy group $(P = 0.21)$. The average age of the IMN group was higher than that of the non-membranous nephropathy group $(P = 0.01)$.

3.2 Comparison of anti-PLA2R antibody (RU mL–1) between IMN and non-membranous nephropathy

The serum PLA2R antibody titer in the IMN group was significantly higher than that in the non-membranous nephropathy group $(H = 235.448, P < 0.001)$. The results are shown in **Table 1**.

Table 1. Comparison of anti-PLA2R antibody (Ru mL^{-1}) between patients with IMN and non-membranous nephropathy

Patients	M (Min, Max)	H	P
IMN	66.80 (0.60, 691.46)	235.448	<0.001
Non-membranous nephropathy	0.61 (0.60, 8.89)		
Control	0.615 (0.60, 2.35)		

3.3 Analysis of general clinical characteristics of IMN in different pathological stages

The age, cys c and Scr in stage III membranous nephropathy group were higher than those in stage I membranous nephropathy group and stage II membranous nephropathy group (P values were

0.003, 0.000 and 0.000, respectively); the titer of serum anti PLA2R antibody in stage II membranous nephropathy group and stage III membranous nephropathy group was higher than that in stage I membranous nephropathy group $(P = 0.006)$. The results are shown in **Table 2.**

Table 2. Analysis of general clinical characteristics of IMN in different pathological stages

Clinical characteristics	Stage I	Stage II	Stage III	Test statistics	P
Number of cases	19	117	19		
Sex (M/F)	13/6	73/44	13/6	0.425	0.798
Age (years)	49.89 ± 9.75	49.26 ± 10.35	57.63 ± 6.28	5.898	0.003
Cys c (mg·L^{-1})	0.97 ± 0.15	1.00 ± 0.29	1.48 ± 0.58	18.208	0.000
Proteinuria (g/24 h)	5.17 ± 3.34	5.63 ± 3.25	5.39 ± 2.94	0.189	0.828
Alb (g·L^{-1})	26.29 ± 8.08	25.64 ± 6.32	27.41 ± 4.24	0.667	0.515
CHO (mmol·L^{-1})	8.18 ± 2.79	7.84 ± 2.46	7.54 ± 1.29	0.349	0.706
Scr (μmol·L^{-1})	75.32 ± 11.09	74.85 ± 25.63	99.67 ± 30.15	8.195	0.000
anti-PLA2R (RU·mL^{-1})	18.96 (0.75, 66.00)	135.90 (0.60, 691.46)	128.9 (2.88, 526.20)	10.261	0.006

3.4 Spearman correlation between anti-PLA2R antibody and clinical indexes in patients with IMN

Serum PLA2R antibody was negatively correlated with Alb ($r_s = -0.234$, $P = 0.003$) with statistical significance, and had no correlation with clinical indexes such as cys c, CHO, Scr and proteinuria ($P = 0.432, 0.704, 0.526$ and 0.078 respectively). The results are shown in **Table 3**.

Table 3. Spearman correlation analysis between anti-PLA2R antibody and clinical indexes in patients with IMN

Clinical characteristics	r_s	P
Cysc (mg L^{-1})	−0.064	0.432
Proteinuria (g/24 h)	−0.142	0.078
Alb (g L^{-1})	−0.234	0.003
CHO (mmol L^{-1})	−0.031	0.704
Scr (μmol·L^{-1})	−0.051	0.526

4. Discussions

With the development of global society, economy and environment, the incidence rate and detection rate of IMN also show a rising trend[6], which is one of the important reasons for chronic kidney disease and progression to end-stage renal

disease. The exploration of IMN's etiology and complete exposition of its molecular biological pathogenesis has never stopped. In 1959, Heymann et al.[7] established a Heymann nephropathy model similar to the pathological changes of human membranous nephropathy in rats. In 2009, Beck et al.[3] found that anti-PLA2R antibody existed in the blood of most IMN patients by Western blotting and mass spectrometry and inferred that a glycoprotein M-type PLA2R with a molecular weight of 185 kD on podocytes of IMN patients is the main target antigen combined with it, but no anti-PLA2R antibody is detected in the blood of non IMN patients, which suggests that anti-PLA2R antibody is a unique molecular biological change of IMN. Through these important studies, The consensus that IMN is an organ specific autoimmune disease has become more and more clear and rich, and further research has been carried out on the detection methods and value of these target antigens and their autoantibodies, especially serum anti-PLA2R antibody and renal PLA2R have become a global research hotspot in recent years.

There are different conclusions on the correlation between serum PLA2R antibody titer and IMN disease activity at home and abroad. Most research results tend to believe that that there is negative correlation between serum PLA2R antibody and Alb. A German study[8] shows that patients with high serum PLA2R antibody titer are often older, and the Alb level was also lower; Kei et al.[9] detected the serum PLA2R antibody in 38 patients with IMN and 21 patients with secondary membranous nephropathy by enzyme-linked immunosorbent assay and indirect immunofluorescence detection at the same time. It was found that the Alb level was negatively correlated with the serum PLA2R antibody titer ($r = –0.468$, $P = 0.043$), but not with the quantification of proteinuria; Yun et al.[10] observed 100 IMN patients and found that the initial Alb level of patients with positive serum PLA2R antibody was significantly lower than that of patients with negative serum PLA2R antibody (2.5 g·DL^{-1} vs 3.1 g·DL^{-1}, $P = 0.004$). The proportion of proteinuria within the scope of nephrotic syndrome in the serum antibody positive group was also significantly higher than that in the serum an-

tibody negative group (87.0% vs 48.4%, $P = 0.001$), but there was no significant difference in renal function between the two groups. Qin et al.[11] found that serum PLA2R antibody was detected by enzyme-linked immunosorbent assay in a study including 572 patients diagnosed as IMN through renal biopsy. Correlation analysis showed that serum antibody titer was negatively correlated with Alb level ($r = –0.20$, $P < 0.001$), but Ramachandran et al.[12] found that there was no significant correlation between PLA2R staining in renal tissue and serum PLA2R antibody titer ($r = 0.03$, $P = 0.76$), there is also no correlation between serum antibody titer and proteinuria and serum albumin. Radice et al.[13] found a positive correlation between serum PLA2R antibody level and proteinuria quantification. However, Julien et al.[14] did not find a correlation between serum PLA2R antibody titer and proteinuria amplitude through retrospective analysis of the clinical data of 68 patients with IMN pathologically diagnosed by renal biopsy. In terms of correlation, Zhou Guangyu et al.[15] studied 15 patients with IMN and found that serum PLA2R antibody was positively correlated with proteinuria and negatively correlated with blood ALB, while Yuan Li et al.[16] analyzed the antibody titer and laboratory related data of 17 patients with IMN with positive serum PLA2R antibody and found that there was no correlation between anti PLA2R antibody and proteinuria and renal function Spearman correlation analysis between anti-PLA2R antibody and clinical indexes in IMN patients in this study suggests that serum anti-PLA2R antibody and Alb was negatively correlated, and has no correlation with cys c, CHO, Scr and proteinuria, the reasons of which may be as follows. (1) Although anti-PLA2R antibody is an important serum marker of IMN, it is only a part of the molecular biology link of the specific pathogenesis of IMN patients, which reflects the immunological activity of patients to a certain extent, or it is only a initiating factor. The production of proteinuria in IMN patients is mainly related to glomerular podocytes. It is related to the destruction of basement membrane structure and functional integrity. For IMN patients with positive serum PLA2R antibody, a very complex molecular bio-

logical stimulation reaction chain is required from the synthesis and secretion of serum PLA2R antibody to the formation of proteinuria. This process also involves some other cells and molecules other than anti-PLA2R antibody. (2) The interval of the renal biopsy in different patients is in different stages of its natural course of diseases, and bias may occur when the sample size of the study is small. (3) The quantitative determination of 24-hour proteinuria is affected by many factors such as urinary volume and glomerular filtration rate. Hypoproteinemia is often more prominent in patients with serious clinical conditions. At this time, due to the low osmotic pressure of plasma colloid, the effective circulating blood volume is also reduced, with reduced blood perfusion of kidney, and the 24-hour urine volume of these patients may also be significantly reduced. So the quantitative urine protein can not well reflect their disease activity, and the Alb index is relatively affected by fewer factors, so it can more accurately reflect the severity of clinical diseases.

The analysis of the general clinical characteristics of IMN in different pathological stages showed that the age, cys c and serum Scr in stage III membranous nephropathy group were higher than those in stage I membranous nephropathy and stage II membranous nephropathy group (*P* values were 0.003, 0.000 and 0.000, respectively); the serum PLA2R antibody titer of stage II membranous nephropathy and stage III membranous nephropathy was higher than that of stage I membranous nephropathy (*P* = 0.006), which was basically consistent with the observation results of some previous studies[17,18]. For patients with IMN, especially those with membranous nephropathy related to anti-PLA2R antibody, the serum PLA2R antibody titer is related to the stage of renal pathological progress. Patients with low serum antibody titer often correspond to the pathological changes of early membranous nephropathy. The serum PLA2R antibody detected in this study is IgG4. Although from the half-life and fluctuation law of IgG4, the low titer level may be at two different pinots of time of the same concentration in the early stage of antibody synthesis and the late stage of elimination. Both early membranous nephropathy and late

membranous nephropathy in the pathological progression stage of membranous nephropathy may correspond to the low titer level of serum antibody, but patients with pathological late membranous nephropathy often have clinical symptoms and signs and are detected, and have even been treated for a period of time. Therefore, for newly hospitalized IMN patients, when the serum PLA2R antibody titer is low, the renal pathology is more likely to be early membranous nephropathy. It can be inferred that the serum PLA2R antibody titer has a certain predictive value for the renal pathological development stage of newly hospitalized IMN patients, especially early membranous nephropathy. Theoretically, there are certain rules from antigen stimulation to antibody synthesis and secretion and onset time, patient body surface area or body weight, IgG half-life, kidney adsorption capacity and swelling curve characteristics of PLA2R antibody. Based on these rules, it is also possible to infer the pathological progression stage of patients with membranous nephropathy by detecting the titer level of serum PLA2R antibody. Because the sample size of this study is too small, and there are no patients with stage IV and V membranous nephropathy included in the study, more cases and patients including all pathological stages are needed as the research objects, and the establishment of a biological and mathematical model based on the half-life and inflation law of PLA2R antibody in blood and the relationship between quantitative and qualitative change of immune complex in the process of kidney deposition gives the possibility to further explore the exact relationship between the quantitative change of serum PLA2R antibody and the progression stage of nephrosis in patients with IMN.

Therefore, the conclusion of this study is that the baseline titer of serum PLA2R antibody in patients with IMN is negatively correlated with serum albumin, and may be related to the pathological progression stage of membranous nephropathy. The titer of serum PLA2R antibody in patients with stage I IMN is significantly lower than that in patients with stage II and III IMN. Limited to conditions, this study is a single center with a small sample size research, its limitations are inevitable. We

expect a larger sample size and multicenter research to provide more powerful evidence.

Conflict of interest

The authors declare no potential conflicts of interest.

References

1. Wang H. Nephrology (in Chinese). 3rd ed. Beijing: People's Medical Publishing House; 2008. p. 1032–1042.

2. Lai W, Yeh T, Chen P, *et al.* Membranous nephropathy: A review on the pathogenesis, diagnosis, and treatment. Journal of the Formosan Medical Association 2015; 114(2): 102–111.

3. Beck LH, Bonegio RG, Lambeau G, *et al.* M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. The New England Journal of Medicine 2009; 361(1): 11–21.

4. Qin W, Beck LH, Zeng C, *et al.* Anti-phospholipase A2 Receptor antibody in membranous nephropathy. Journal of the American Society of Nephrology 2011; 22(6): 1137–1143.

5. Ehrenreich T, Chung J. Pathology of membranous nephropathy. Patho Annu 1968; 3: 145–186.

6. Couser WG. Primary membranous nephropathy. Clinical Journal of the American Society of Nephrology Cjasn 2017; 12(6): 983.

7. Heymann W, Hackel DB, Harwood S, *et al.* Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspensions. Experimental Biology & Medicine 1959; 100: 660–664.

8. Hoxha E, Harendza S, Pinnschmidt H, *et al.* M-type phospholipase A2 receptor autoantibodies and renal function in patients with primary membranous nephropathy. Clinical Journal of the American Society of Nephrology Cjasn 2014; 9(11): 1883–1890.

9. Kei H, Masayuki I, Shohei T, *et al.* Anti-phospholipase A2 receptor (PLA2R) antibody and glomerular PLA2R expression in Japanese patients with membranous nephropathy. Plos One 2016; 11(6): e0158154.

10. Yun J, Yang S, Dong K, *et al.* Autoantibodies against phospholipase A2 receptor in Korean patients with membranous nephropathy. Plos One 2013; 8(4): e62151.

11. Qin H, Zhang M, Le W, *et al.* Combined assessment of phospholipase A2 receptor autoantibodies and glomerular deposits in membranous nephropathy. Journal of the American Society of Nephrology 2016; 27(10): 3195–3203.

12. Ramachandran R, Kumar V, Kumar A, *et al.* PLA2R antibodies, glomerular PLA2R deposits and variations in PLA2R1 and HLA-DQA1 genes in primary membranous nephropathy in South Asians. Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplantation Association-European Renal Association 2016; 31(9): 1486–1493.

13. Radice A, Trezzi B, Maggiore U, *et al.* Clinical usefulness of autoantibodies to M-type phospholipase A2 receptor (PLA2R) for monitoring disease activity in idiopathic membranous nephropathy (IMN). Autoimmunity Reviews 2016; 15(2): 146–154.

14. Jullien P, Barbara SP, Maillard N, *et al.* Anti-phospholipase A2 receptor antibody levels at diagnosis predicts spontaneous remission of idiopathic membranous nephropathy. Clinical Kidney Journal 2017; 10(2): 209–214.

15. Zhou G, Jin L, Yu J, *et al.* Correlation between serum anti PLA2R antibody and disease condition in adult patients with membranous nephropathy (in Chinese). Chinese Journal of Nephrology 2012; 28(2): 111–114.

16. Yuan L, Shi L, Xu Y, *et al.* Clinical significance of anti-M type phospholipase A2 receptor antibody in treatment of idiopathic membranous nephropathy. Chinese Journal of Clinical Laboratory Science 2015; 33 (8): 581–585.

17. Ping Z, Fu D Z, Su X, *et al.* Increasing frequency of idiopathic membranous nephropathy in primary glomerular disease: A 10-year renal biopsy study from a single Chinese nephrology centre. Nephrology 2015; 20 (8): 560–566.

18. Cattran DC. Idiopathic membranous glomerulonephritis. Kidney International 2001; 59(5): 1983–1994.