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ORIGINAL RESEARCH

Expression of phospholipase A2 receptor and IgG4 in patients with membranous nephropathy

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Objectives: The aims of this study were to detect the expression of M phospholipase A2 receptor (PLA2R) in the kidney tissue of patients with idiopathic membranous nephropathy (IMN), secondary membranous nephropathy (SMN), and the nonmembranous nephropathy (non-MN), to evaluate the value of PLA2R in the kidney tissue and serum anti-PLA2R antibody in the diagnosis of membranous nephropathy (MN), and to explore the relationship between PLA2R of the kidney tissue or serum anti-PLA2R antibody and clinical features of MN.

Methods: The kidney tissue was collected by kidney biopsy. Immunofluorescence assay was used to detect the level of PLA2R and IgG4 antigen in kidney tissue. Furthermore, the level of the PLA2R was detected using the enzyme-linked immunosorbent assay (ELISA). The positive and negative rates of PLA2R and IgG4 in different diseases and the sensitivity and specificity, were calculated using the statistical method. The specificity and coincidence rate of PLA2R or anti-PLA2R used in the differential diagnosis of IMN and SMN were evaluated.

Results: The expression intensities of anti-PLA2R antibody and IgG4 were significantly higher in patients with IMN than in patients with SMN but are not non-MN. There was no significant difference in anti-PLA2R antibody and IgG4 in patients with SMN and non-MN. The coincidence rate of serum anti-PLA2R antibody and PLA2R in kidney tissue was 100%.

Conclusion: The expression of PLA2R and IgG4 antibody had great significance in the pathological diagnosis of MN. The detection of the serum anti-PLA2R antibody had great diagnostic value in diagnosing MN.

Keywords: membranous nephropathy, PLA2R, IgG4

Introduction

Membranous nephropathy (MN) is one of the most common pathological types of the nephrotic syndrome in China. With the gradual improvement of the utilization rate of pathological diagnosis technology in the diagnosis of kidney diseases, the increasing incidence of MN has been reported in many different medical centers[1] and has become the most common cause of nephrotic syndrome.

Usually, the MN refers to the idiopathic MN (IMN), which is also named the primary MN. Approximately 80% of patients were diagnosed with nephrotic syndrome characterized by large number of proteinuria and hypoalbuminemia, complicated with infection, thrombosis, and embolism events. Without a timely and effective diagnosis and treatment, patients may progress to end-stage renal failure after 5–10 years. Therefore, it is of high urgency to improve the diagnosis and treatment effect.

In the past many authors have studied how to improve the accurate diagnosis rate of MN. In 2009, Beck et al[2] first discovered that the extracellular domain of phospholipase

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A2 receptor (PLA2R) was used as the mutant antigen to activate autoimmunity response. Combined with the PLA2R antibodies produced in the body, PLA2R forms the insitus immune complex, resulting in the injury of basement membrane, which was the major pathogenic factor of the majority of IMN patients. Many literature reported that the detection of peripheral blood in patients with serum, results positive rate of anti PLA2R antibodies in patients with IMN was 50–80%,[3–5] and was about 20%[6] in patients with secondary MN (SMN), in the patients with non-MN and normal population was almost to zero.[7] Recent studies have pointed out that the titers of anti-PLA2R antibodies in peripheral blood were associated with the disease activity.[8–10] Thus, the detection of serum anti-PLA2R antibodies had great significance for diagnosis, treatment, and prognosis of the disease.

In recent years, more and more studies have confirmed that the immunoglobulin IgG subtypes play an vital role in the identification of MN caused by different antibodies, such as IgG4 mainly in IMN kidney tissue deposits[11] and IgGl and IgG3 mainly in SMN.[12,13] Beck et al[2] first identified IgG4 as the major component of anti-PLA2R antibody in IMN patients, which is consistent with kidney pathology. Thus, the detection of glomerular IgG4 expression could serve as the reference index in the diagnosis and differential diagnosis of kidney diseases. In this study, we analyzed the data of patients with MN diagnosed in our hospital and the expression of PLA2R and IgG subtype in blood and kidney tissue, respectively. The presence of IgG subtype and PLA2R in the identification of IMN and non-MN was assessed, and the values of both expressions were also evaluated.

Methods

Patients

A total of 39 (including 27 males and 12 females) patients who underwent renal biopsy were selected from the Department of Nephrology, The First Affiliated Hospital of Bengbu Medical College, Bengbu, China, from August 2016 to December 2016. Signed informed consent was obtained from all the patients. This study was approved by the ethics committee of The First Affiliated Hospital of Bengbu Medical College (2016094).

Inclusion and exclusion criteria for patients

Patients aged between 12 and <65 years and those who satisfied the standard diagnostic criteria of chronic kidney disease (CKD) were included in the study. The diagnosis of CKD was made when one of the following two criteria was met: 1) structural or functional abnormalities of the kidney lasting for at least 3 months, and 2) glomerular filtration rate (GFR) <60 mL/min/1.73 m^2 for at least 3 months.[14] Patients were excluded based on the following two criteria: 1) patients who were not willing to perform kidney pathology, and 2) patients who had contraindications to kidney pathology. The risk of the kidney pathology examination is higher in patients older than 65 years, hence, the examination was not done in these patients.

Inclusion and exclusion criteria for IMN and SMN

The inclusion criteria for IMN were as follows: the pathological diagnosis of the kidney tissue was MN, except for clinical factors such as systemic lupus erythematosus (SLE), hepatitis B virus (HBV), tumor, and drug-induced secondary membranous nephropathy. The inclusion criteria for SMN were as follows: 1) membranous lupus nephritis (MLN): ≥ SLE classification revised by Systemic Lupus International Collaborating Clinics (SLICC) in 2012 and the pathological diagnosis of the kidney tissue was MN; 2) HBV-related glomerulonephritis (HBV-GN): serum HBsAg was positive, except for clinical factors such as SLE, tumor, and drugs, the pathological diagnosis of the kidney tissue was MN, and hepatitis B surface antigen (HBsAg) and/or hepatitis B core antigen (HBcAg) in the kidney tissue were positive.[15]

Clinical data

Age, gender, serum creatinine, serum albumin, and 24 h protein excretion were used for this study.

Kidney histopathology examination

The kidney tissues were collected from the patients, and the pathological specimens examined under light immunofluorescence microscopy were sent to KingMed Diagnostics.

Kidney pathology and immunofluorescent examination

Immunofluorescent staining was performed for PLA2R and IgG4 on 3 μm sections of formalin-fixed tissue using a kit, according to manufacturer's instructions. Briefly, the rabbit antihuman PLA2R polyclonal antibody (1:100 dilution; Sigma-Aldrich Co., St Louis, MO, USA) was added to the sections. After 1 h of incubation, the goat antirabbit IgG (Abcam, Cambridge, UK), which had been diluted to 1:50, was added to the sections. After 1 h of incubation, the sections were washed with water, dried, glycerol mounted, and then observed under fluorescence microscopy. The method and steps of IgG4 detection were as follows: the first antibody

mouse antihuman CD138-FITC (Maixin, FuZhou, China) and the second antibody (1:200 dilution) IgG4 monoclonal antibody (Abcam) were added to the sections. After 1 h of incubation, the sections were washed with water, dried, glycerol mounted, and then observed under fluorescence microscopy. Fluorescence intensity determination: observed by fluorescence microscopy, -, negative, ±/+~ 3 +, positive.[16]

Serum anti-PLA2R antibody detection

According to the kit instructions (Euroimmun, Lübeck, Germany), the blood was extracted from the peripheral vein and the serum was detected by ELISA after centrifugation. The ELISA detector measured the absorbance value at the 450 nm wavelength. The serum antibody level was calculated according to the curve equation, with 0–14 RU/mL as the standard reference value of the serum antibody.

Statistical analysis

Normally distributed data are presented as the mean ± standard deviation. The data were statistically analyzed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). Parametric data were analyzed using one-way analysis of variance and Student's *t*-test. The sensitivity and specificity, negative predictive value, and positive predictive value were calculated by Fisher's exact test. $P<0.05$ was considered as a statistically significant difference.

Results

Clinical data and biochemical parameters

There were 39 patients enrolled in this study from August 2016 to December 2016. All patients underwent kidney biopsy, including 27 males and 12 females, with mean age 45.8±13.6 years, and were divided into the following three groups according to the pathological results: primary MN group (IMN; 27 patients), SMN group (five patients), and non-MN group (seven patients). No statistically significant differences were observed for serum albumin and 24 h urine protein in the three groups. There was one case of acute renal injury and one case of severe lupus in the non-MN group; the serum creatinine concentration of two patients was significantly higher than normal, so there was a significant difference compared with IMN and SMN groups ($P<0.01$) (Table 1).

PLA2R and IgG4 expressions in the kidneys of the three groups

The positive expressions of PLA2R (88.89%) and IgG4 (81.48%) were higher in the IMN group than in the SMN group and non-MN group, and the difference was statistically significant ($P<0.01$). There was no significant difference in PLA2R-positive expression and IgG4-positive expression between the SMN group and non-MN group ($P>0.05$) (Table 2).

Comparison of PLA2R between kidney tissue and serum in IMN patients

The results showed that 24 patients whose kidney tissue PLA2R were positive and their anti-PLA2R antibodies in serum were also detected positive. Three cases of IMN kidney tissue PLA2R-negative patients with anti-PLA2R antibodies in serum were also negative, suggesting that serum

Table 1 Clinical data and biochemical parameters ($\bar{x} \pm s$)

Group	n	Scr (μmol/L)	ALB (g/L)	24 h U-pro (g)
IMN	27	73.89±19.17	23.77±7.10	5.06±2.38
SMN	5	63.60±10.38	22.34±3.27	5.49±2.19
Non-MN	7	273.86±289.66*	30.21±9.02	5.56±2.40
F		8.198	2.580	0.169
P		0.001	0.090	0.845

Notes: The clinical data and biochemical parameters between the IMN and SMN groups showed no differences. Scr in the non-MN group was higher than that in the IMN and SMN groups; the reason was that several pathological diagnoses in the kidney tissue were FSGS, glomerulosclerosis, and tubulointerstitial injury and the kidney function had been significantly damaged. *IMN and SMN compared with non-MN, $P<0.05$. Data is presented as mean ± SD.
Abbreviations: ALB, albumin; FSGS, focal segmental glomerulosclerosis; IMN, idiopathic MN; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; Scr, serum creatinine; SMN, secondary MN; U-pro, urine protein.

Table 2 PLA2R and IgG4 expressions in the kidneys of the three patient groups, n (%)

Groups	PLA2R(+)	IgG4(+)
IMN (n=27)	24 (88.89)	22 (81.48)
SMN (n=5)	2 (40.00)*	1 (20.00)*
Non-MN (n=7)	0 (0.00)*	1 (14.29)*
x^2	24.081	15.349
P	0.000	0.000

Notes: The result was consistent with previous studies. *Compared with the IMN group, the expression of PLA2R and IgG4 in the kidneys of SMN and non-MN groups was not obvious, $P<0.05$.
Abbreviations: IMN, idiopathic MN; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; SMN, secondary MN.

Table 3 Comparison of PLA2R between kidney tissue and serum in IMN patients

Serum anti-PLA2R antibody	Kidney tissue PLA2R	
	Positive	Negative
Positive	24	0
Negative	0	3
Total	24	3

Note: The diagnostic specificity of PLA2R for IMN has not reached 100%, which is consistent with related reports.
Abbreviations: IMN, idiopathic membranous nephropathy; PLA2R, phospholipase A2 receptor.

anti-PLA2R antibody and kidney tissue PLA2R detection consistent rate was 100% (Table 3).

Evaluation of indicator of PLA2R and IgG4 expressions in the kidney tissue

According to the results, the detection of the PLA2R and IgG4 expression in the kidney tissue had higher sensitivity compared with SMN group and non-MN groups. The detection of the PLA2R or IgG4 had higher specificity compared with SMN group and non-MN groups, which may be due to the specificity of expression of PLA2R in the non-IMN group. Compared with the sensitivity, specificity, negative predictive value, positive predictive value, and coincidence rate in the three groups, no statistically significant difference was observed (P=0.459, 0.222, 0.387, 0.718, and 0.843). This was a significant limitation in our study; the number of patients included was small due to the shorter research period. We will include more cases in the future to improve research persuasiveness (Table 4).

Immunofluorescence changes in the three groups

Immunofluorescence showed that there were significant differences in the fluorescence values among the three groups (Figure 1). Immunofluorescence-labeled immunocomplex was more abundant in the glomerular capillary loops in the IMN group but not in the SMN and non-MN groups.

Discussion

MN is a common pathological type of nephrotic syndrome, and the incidence increases year by year. Some causes such as the hepatitis, drug, high incidence of cancer, environmental pollution and so on could cause MN.[17–20] In the present study, we collected all patients who underwent kidney pathology within 5 months in our hospital were collected; the statistical results showed that the incidence of MN was up to 69.2%, and the incidence of MN was higher than IgA nephropathy, which had been the first cause in chronic glomerular disease.

Phospholipase A2 (PLA2) is a group of enzymes distributed in multiple organs of the human body. It has many subtypes and can bind to PLA2R to form an immune complex, which plays a key role in regulating cell proliferation, adhesion, and activity factor secretion in the inflammatory reaction process. PLA2R was overexpressed in renal tissue of IMN patients, and anti-PLA2R antibody produced by its expression was also observed. A series of studies confirmed that anti-PLA2R antibody levels in IMN patients were significantly elevated compared with the normal and non-IMN patients.[21,22] Anti-PLA2R antibodies bind to PLA2R on kidney podocytes to cause complement activation, podocyte damage, and basement membrane damage.[23,24] In this study, we found that the PLA2R from kidney tissue was positive in 88.89% of IMN patients, while PLA2R from kidney tissue was positive in 40 and 0% of SMN and non-MN patients, respectively. Beck et al reported that IgG4 was mainly anti-PLA2R antibodies in IMN patients. Many studies showed that the pathogenesis of IMN is because the recognition of IgG4 recognition and the glomerular podocyte PLA2R, the formation of in situ immune complexes, and the activation of the complement system, which led to immune injury, podocyte morphological change, proteinuria, hypoproteinemia, and other clinical symptoms.[25] Our study showed that the positive rate of IgG4 expression in kidney tissue of IMN patients was 81.48%, while the positive rate of IgG4 expression in kidney tissue of SMN and non-MN patients was 20 and 14.9%, respectively.

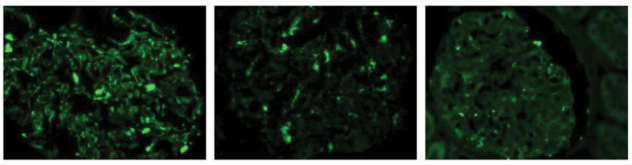

Figure 1 Fluorescence values among the three groups.
Notes: The fluorescent-labeled goat antihuman IgG was combined with PLA2R, which expressed on the glomerular capillary filtration membrane, and then formed the immunocomplex. Under immunofluorescence microscope, there had a significant difference expression in three groups. Magnification ×400.
Abbreviations: IMN, idiopathic MN; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; SMN, secondary MN.

Table 4 Evaluating indicators of PLA2R and IgG4 expressions in the kidney tissue, n (%)

Groups	IMN		Non-IMN		Sensitivity	Specificity	+PV	–PV	Coincidence rate
	Yes	No	Yes	No					
PLA2R+	24	3	2	10	88.89	83.33	92.31	76.92	87.18
IgG4+	22	5	2	10	81.48	83.33	91.67	66.67	82.05
PLA2R+ and IgG4+	26	1	4	8	96.30	66.67	86.67	88.89	87.18
PLA2R+ or IgG4+	23	4	0	12	85.19	100.00	100.00	75.00	89.74

Notes: The sensitivity and negative predictive value in group PLA2R+ and IgG4+ were higher compared with other groups. The specificity and coincidence rate in group PLA2R+ or IgG4+ were higher compared with other groups, but there was no statistical difference (P>0.05).
Abbreviations: IMN, idiopathic membranous nephropathy; PLA2R, phospholipase A2 receptor; PV, predictive value.

However, there were two cases of SMN positive for PLA2R and one case of SMN and non-MN positive for IgG4. This may result from: 1) there was a possibility of IMN combined with another disease;[26] 2) the combined detection of PLA2R and IgG4 could not completely exclud the secondary causes; and 3) the mechanism of MN was not completely clear and the explanation of the role of PLA2R and IgG4 in patients with MN was difficult.[27] Still, we have reasons to believe that the positive rate of IgG4 in kidney tissue consistent with PLA2R, and the detection of IgG4 combined with PLA2R improved the diagnostic accuracy of MN. Therefore, IgG4-related disease (IgG4-RD) is recently gaining a lot of attention. It is worth mentioning that IgG4 found in IMN is not IgG4-related disease. In 2012, IgG4-related MN was proposed by Alexander et al.[28] In patients with MN secondary to IgG4-RD, an immunofluorescence assay showed granular deposits of C3 and IgG, of which IgG4 was the dominant subclass, along the glomerular basement membrane. In these patients, the kidney tissue is typically negative when staining with anti-PLA2R antibodies, similar to the serum anti-PLA2R staining. Currently, there is still no uniform diagnostic standard for MN secondary to IgG4-RD. The diagnosis of MN secondary to IgG4-RD should be made in the context of IgG4-RD in other organs or IgG4-related tubulointerstitial nephritis (IgG4-TIN).[29]

Conclusion

IgG4 and PLA2R detection in kidney tissue was used as an important diagnostic tool. For those patients who were not or refused to perform kidney pathology, anti-PLA2R antibody detection in serum was used as a convenient and rapid detection method to provide guidance for clinical diagnosis. In this study, the number of patients included was less because of the shorter period of study. We will continue this study and include more patients to improve the scientific nature of this study.

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Disclosure

The authors report no conflicts of interest in this work.

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