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Original Article

Clinicopathological features of idiopathic membranous nephropathy in patients of different ages

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Abstract: Idiopathic membranous nephropathy (IMN) is increasingly seen in elderly patients, however, the clinicopathological features of IMN are still elusive. This study aimed to compare and analyze the clinicopathological features among IMN patients of different ages. In this study, a total of 142 IMN patients were recruited and grouped by age. After comparing and analyzing their clinical and pathological data, we found that (1) The course of disease in the elderly group was longer, but not significantly (P=0.834). The glomerular filtration rate (eGFR), triglyceride and hemoglobin levels of the elderly group were significantly lower than those of the young and middle-aged groups (P=0.001, P=0.021, P=0.031), and the incidence of nephrotic syndrome was significantly higher than the other two groups (P=0.026). (2) By immunofluorescence, deposition in the glomerulus was shown with IgG4 (88.03%) and C3 (97.89%) predominance. The mild glomerular sclerosis (82.05%), tubule atrophy (92.31%) and renal arterial wall thickening (25.64%) in the elderly group were much higher than those in the young (44.74%, 63.16%, 2.63%) and middle-aged groups (60%, 83.08%, 18.46%), which is statistically significant (P=0.001, P=0.004, P=0.016). (3) The serum PLA2R antibodies in IMN patients was negatively correlated with the level of albumin (P=0.001), whereas there was no significant correlation between PLA2R and 24 h urine protein (P=0.180). In conclusion, elderly IMN patients are more likely to be accompanied by nephrotic syndrome with more serious pathological kidney damage. PLA2R level is negatively correlated with serum albumin, but has no correlation with 24 h urine protein.

Keywords: Idiopathic membranous nephropathy, clinical manifestations, pathological features, M-phospholipase A2 receptor antibody, IgG4

Introduction

Membranous nephropathy (MN) is one of the most common pathological types of nephrotic syndrome in adults, which is usually mediated by immune complexes. It is common in adults, especially in elderly patients, but rarely found in children [1]. Every year about 12 people per one million adults are diagnosed with MN worldwide [2, 3]. Without standardized treatment, end-stage renal disease (ESRD) will happen in at least one-third of patients within 15 years [4]. To date, the diagnosis of MN mainly based on pathological examination of kidney biopsy, which is characterized by the thickening of glomerular basement membrane (GBM) caused by the aggregation of immune complexes, which is observed as granular deposits of immunoglobulin G (IgG) on the GBM under immunofluorescence. In addition, its main clinical symptoms are edema and heavy proteinuria [5].

Accumulating evidence has shown that MN is a common pathological type of glomerular disease in the elderly [6], accounting for approximately 12.6% of patients with primary kidney disease that undergo renal biopsy [7], which increases every year [8]. Clinically, MN can be divided into two categories according to the etiology: idiopathic membranous nephropathy (IMN) and secondary membranous nephropathy (SMN). IMN is the main type and accounts for about 80% of MN. It is one of the main causes of adult nephrotic syndrome, and is also the glomerular disease that recurs most frequently after kidney transplantation (about 40%) [2]. Generally, IMN happens in older peo-

ple, but its incidence has significantly increased in China recently according to the large-scale epidemiological data, which is mainly due to the increase in younger patients (14 to 44 years old) [9]. IMN, as an antibody-mediated autoimmune glomerular disease, is characterized by subcutaneous immune deposition. It usually has a long course of disease and is prone to induce acute kidney injury, which finally becomes chronic kidney disease and even leads to death [10]. Immune complex deposition, glomerular basement membrane thickness and podocyte morphological changes are the causes of proteinuria [11]. However, the pathogenesis of IMN has not been fully elucidated [12]. At present, it is mainly accepted that the formation of immune complexes under glomerular epithelial cells is associated with specific endogenous antigens of podocyte and their corresponding antibodies. Phospholipase A2 receptor (PLA2R) is one of its main antigens, which is highly related to the occurrence, development and prognosis of IMN. It is reported that the deposition of immunoglobulin IgG4 on GBM is commonly detected in IMN which accounts for 7% of immunoglobulin G4-related diseases (IgG4-RDs), and IgG4-related IMN occurs mainly in male patients [13]. Recently, several autoantigens, including secreted M type PLA2R, thrombospondin type 1 domain-containing 7A (THSD7A), and neural epidermal growth factor-like 1 protein (NELL-1), have been identified in IMN. Therefore, the determination of peripheral antibodies has become an important clinical reference index. However, some clinical features of IMN are still elusive and further research on topics such as autoimmune initiation, IgG4 predominance, spontaneous remission, and unique glomerular disease is required [14]. Accordingly, it is very important to clarify the different clinicopathological characteristics between young and old patients with IMN, whereas the research on IMN in young people until now is still very limited and controversial. Based on this, our study was designed to summarize and analyze the clinical and pathological features of IMN patients of different ages.

Methods

Study population

The present study included 142 patients that underwent renal biopsies under ultrasound guidance and were diagnosed as having IMN from January 2014 to March 2020 in the Department of Nephrology, Shenmu Hospital Affiliated to Northwest University.

Patients with systemic lupus erythematosus, hepatitis B, tumors and infectious diseases were already pre-excluded. The clinical and pathological data of all cases were completely saved and anonymized prior to accessing the database. The patients were grouped by age.

Ethics approval and consent to participate

All participants were informed of what was expected from them and their rights. Written informed consent was obtained from each participant for blood sampling and renal biopsy during their hospitalizations. This study was approved by the Ethics Committee of Shenmu Hospital Affiliated to Northwest University (Figure S1).

Clinical and laboratory data

Data collection: Demographic data and laboratory data were collected at the time of biopsy. Baseline information at the time of biopsy included age, sex, course of disease (from the first appearance of clinical manifestations including edema and urine protein to renal biopsy), history of hypertension and diabetes, systolic and diastolic blood pressures, clinical manifestations, etc. Additionally, height, weight, and body mass index (BMI) were also calculated. The laboratory data included 24 h urinary protein, hematuria, blood urea nitrogen (BUN), serum creatinine (Scr), uric acid (UA), total protein (TP), albumin (ALB), triglyceride (TG), total cholesterol (TC) and hemoglobin (Hb). The eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation that was GFR (mL/min/1.73 m^2) =141 × min (serum creatinine/*k*, 1)$^{\alpha}$ × max (serum creatinine/*k*, 1)$^{-1.209}$ × 0.993^{Age} × 1.018 (if female) × 1.159 (if black), where *k* is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates minimum serum creatinine/*k* or 1, and max indicates maximum serum creatinine/*k* or 1 [15].

Serum PLA2R-antibody detection: The anti-PLA2R (IgG) test kit from German Oumeng Company was used to detect the titer of

PLA2R antibody in patient's serum through ELISA. The value t the ml was considered as positive [16]. The detection was completed by Xi'an Jinyu Medical Inspection Co., Ltd one week before renal biopsy.

Pathological data: Renal biopsies were performed under the guidance of ultrasound, and the collected tissue was sent to the Shaanxi Provincial Hospital of Traditional Chinese Medicine for examination by light microscopy, immunofluorescence microscopy, and electron microscopy. Glomerular sclerosis, tubular atrophy, tubulointerstitial fibrosis, renal interstitial inflammatory cell infiltration, mesangial cell proliferation, and renal arteriolar sclerosis were examined through HE, PAS, PASM and Masson staining under light microscopy. The deposition of IgA, IgM, C3, C1q, Fib, PLA2R, and IgG subtypes (IgG1, IgG2, IgG3, IgG4) in the glomeruli was detected by immunofluorescence staining.

Diagnostic criteria: (1) Nephrotic syndrome: edema, 24 h urine protein ≥3.5 g/d with hypoproteinemia (Alb <30 g/L). (2) Microscopic hematuria: number of urinary sediment red blood cells ≥100,000/ml. (3) Hypertension: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg twice or more at rest. (4) Hyperuricemia: hyperuricemia is a metabolic syndrome caused by a disorder of purine metabolism. Whether male or female, the blood uric acid level exceeds 420 μmol/L [17].

Statistical analysis

All analysis was performed with SPSS version 26.0. Data was presented as mean ± standard error ($\bar{x}\pm s$). Categorical variables were described as frequencies or percentages. Differences were compared between groups with the Student's t-test, and χ^2 test was used. Differences in mean values were considered significant at $P<0.05$.

Results

General clinical data

In this study, 142 IMN patients aged from 18 to 71 years old were analyzed, including 38 (male 26, female 12) patients as the young group, 65 (male 41, female 24) patients as the middle-aged group, and 39 (male 30, female 9) patients as the elderly group. Baseline characteristics stratified by the three age categories are shown in **Table 1**.

All patients in this study had a course of disease in the range of 1-168 months and showed proteinuria, including 127 (90.1%) with nephrotic syndrome, and 110 (46.5%) with microscopic hematuria. Among them, the elderly group displayed longer course of disease than the young and middle-aged groups, which was not significant (P=0.834), while the incidence of nephrotic syndrome in the elderly group was significantly higher (P=0.026). In addition, the levels of eGFR, triglyceride and hemoglobin in elderly group were much lower comparing to young and middle-aged groups, and the differences were significantly associated with patient age (P=0.001, P=0.021 and P=0.031, respectively). However, the differences among the 3 groups on other parameters including gender, BMI, concomitant hypertension, diabetes, systolic blood pressure, diastolic blood pressure, 24 h urine protein, microscopic hematuria, uric acid (UA), albumin (ALB), total cholesterol (TC) were not statistically significant, as shown in **Table 1**.

Renal histopathological features

Renal biopsy was used for IMN diagnosis, and the representative images of light microscopy, immunofluorescence staining and electron microscopy were shown in Figure S2. The 142 patients with IMN included 18 in stage I (12.7%), 121 in stage II (85.2%), 3 in stage III (2.1%), and there were no patients in stage IV or V. The deposition in the glomerulus was detected by immunofluorescence which was distributed in the granule along the capillary loops; IgG4 (88.03%) and C3 (97.89%), some with IgM (21.83%) and C1q (36.62%), and some with IgA (7.04%) and Fib (5.63%). The ratio of mesangial hyperplasia, and deposition of C1q in middle-aged group were higher than those of elderly group and young group, but the difference was not statistically significant (P=0.428, P=0.393). In addition, the presence of tubulointerstitial fibrosis, renal interstitial inflammatory cell infiltration, C3 deposition, and glomerular PLA2R-ag positive in the elderly group were all higher than those in the middle and young group, but

Table 1. Clinical characteristics of IMN patients in different age groups [n (%), $\bar{x}\pm s$]

Parameter	Young (n=38)	Middle-age (n=65)	Elderly (n=39)	$F/X^2/Z$	*P*
Age (years)	34.11±7.57	51.77±6.02	67.31±4.32	286.49	0.001
Male	26 (68.42)	41 (63.08)	30 (76.92)	2.159	0.340
Diabetes	2 (5.26)	7 (10.77)	9 (23.08)	5.911	0.052
Hypertension	13 (34.21)	26 (40.00)	39 (100)	3.291	0.193
Nephrotic syndrome	31 (81.57)	57 (87.69)	39 (100)	7.299	0.026
Hyperuricemia	15 (39.47)	15 (23.08)	16 (41.03)	4.773	0.092
PLA2R-ab positive	26 (68.42)	40 (61.54)	28 (71.79)	1.261	0.532
Microscopic hematuria	27 (71.05)	47 (72.31)	35 (89.74)	7.540	0.110
Course of disease (months)	20.74±6.40	20.34±4.23	24.87±7.26	0.181	0.834
BMI (kg/m^2)	25.08±3.83	25.46±3.34	24.27±3.49	1.403	0.249
Systolic BP (mmHg)	125.13±19.97	128.06±18.49	135.03±17.33	2.95	0.056
Diastolic BP (mmHg)	87.34±13.61	85.48±11.92	83.56±11.48	0.91	0.404
24 h urine protein (g/d)	4.73±2.11	4.18±1.86	4.39±1.77	0.986	0.376
Serum albumin (g/L)	24.00±7.52	24.50±5.62	22.39±5.33	1.48	0.231
Serum creatinine (μmol/L)	54.12±17.42	55.39±19.05	72.32±37.78	6.74	0.002
eGFR ml/(min·1.73 m^2)	126.97±18.35	110.35±16.95	90.09±25.02	33.49	0.001
PLA2R-ab titer (RU/ml)	89.75±15.65	137.10±29.46	157.24±46.97	0.902	0.408
Uric Acid (μmol/L)	392.18±72.60	376.25±59.53	389.38±84.50	0.76	0.469
Triglyceride (mmol/L)	3.65±2.45	2.24±0.96	2.19±1.23	3.95	0.021
Total cholesterol (mmol/L)	7.39±2.58	7.06±2.16	6.97±1.94	0.404	0.669
Hemoglobin (g/L)	133.26±19.62	136.11±17.74	126.33±17.30	3.57	0.031

PLA2R-ab: phospholipase A2 receptor antibody; BMI: body mass index; BP: blood pressure; eGFR: glomerular filtration rate.

without significant difference (P>0.05). However, mild glomerular sclerosis (82.05%), tubule atrophy (92.31%) and renal arterial wall thickening (25.64%) in the elderly group were all much higher than those in the young group (44.74%, 63.16%, 2.63%) and middle-aged group (60%, 83.08%, 18.46%), which was statistically significant (P=0.001, P=0.004, P=0.016), as shown in **Table 2**.

Correlation of PLA2R antibody with albumin and 24 h urine protein in serum of IMN patients

Through analysis of correlation, we found that the level of PLA2R antibodies in serum was negatively correlated with the level of albumin with the correlation coefficient r=-0.269 (P=0.001), whereas the correlation coefficient between serum anti-PLA2R antibodies and 24 h urine protein was r=0.113 (P=0.180), as shown in **Figures 1** and **2**.

Discussion

MN is one of the most common clinical glomerular diseases with diverse clinical manifestations, of which IMN is the main type with increasing incidence elderly patients. Accordingly, our study retrospectively analyzed and summarized the clinical and pathological characteristics of 142 patients with IMN of different ages in Shenmu Hospital affiliated to Northwest University.

The clinical manifestations of IMN are mainly edema, nephrotic syndrome, hypertension, and microscopic hematuria, while renal failure is rare. In this study, 100% of the patients had proteinuria, and 90.1% showed nephrotic syndrome, which was similar with other reports [2, 18]. In addition, the incidence of hematuria was 46.5%, and mainly manifested as microscopic hematuria, which is in line with the clinical characteristics of IMN. By analyzing the clinical data of each group, we found that compared to young and middle-aged patients with IMN, elderly patients had a relatively longer course of disease, and showed higher proportion of concomitant hypertension and diabetes as well as significantly higher rates of nephrotic syndrome. Furthermore, the incidence of hyperuricemia in the middle-aged group was

Table 2. Pathological features of IMN patients in different age groups [n (%)]

Parameter	Young (n=38)	Middle-age (n=65)	Elderly (n=39)	X^2	*P*
Pathological stage				12.407	0.015
I	4 (10.52)	12 (18.46)	2 (5.13)		
II	31 (81.58)	53 (81.54)	37 (94.87)		
III	3 (7.89)	0	0		
Mesangial cell proliferation				3.84	0.428
None	0	0	0		
Mild	36 (94.73)	63 (96.92)	35 (89.74)		
Moderate	2 (5.26)	2 (3.08)	3 (7.69)		
Severe	0	0	1 (2.56)		
Glomerular sclerosis				13.338	0.01
None	18 (47.37)	24 (36.92)	6 (15.38)		
Mild	17 (44.74)	39 (60)	32 (82.05)		
Moderate	2 (5.26)	1 (1.54)	0		
Severe	0	0	0		
Crescents	1 (2.63)	1 (1.54)	0	0.953	0.621
Tubular atrophy	24 (63.16)	54 (83.08)	36 (92.31)	10.923	0.004
Tubulointerstitial fibrosis	5 (13.16)	5 (7.69)	4 (10.26)	0.816	0.665
Renal interstitial inflammatory cell infiltration	25 (65.79)	53 (81.54)	33 (84.62)	4.795	0.091
Renal arterial wall thickening	1 (2.63)	12 (18.46)	10 (25.64)	8.215	0.016
Fib deposition	3 (7.89)	3 (4.62)	2 (5.13)	0.511	0.775
IgM deposition	10 (26.31)	11 (16.92)	10 (25.64)	1.697	0.428
IgA deposition	3 (7.89)	3 (4.62)	4 (10.26)	1.242	0.537
C3 deposition	36 (94.74)	64 (98.46)	39 (100)	2.769	0.250
C1q deposition	14 (36.84)	27 (41.54)	11 (28.21)	1.868	0.393
IgG subtype deposition				3.092	0.797
IgG1	3 (7.89)	5 (7.69)	6 (15.38)		
IgG2	6 (15.79)	7 (10.77)	7 (17.95)		
IgG3	2 (5.26)	3 (4.62)	1 (2.56)		
IgG4	35 (92.11)	58 (89.23)	32 (82.05)		
PLA2R-ag positive	26 (68.42)	46 (70.77)	33 (84.62)	3.247	0.197

lower than the other two groups, but the kidney function decreased with age. Regarding the 24 h urine protein, serum albumin, and other clinical data of each age group, the differences are not significantly, which might be related to factors such as inclusion and exclusion criteria and sample size [19-21]. Studies revealed that the kidney function of IMN patients at the time of biopsy is an important risk factor affecting prognosis, which is also closely related to hypertension, diabetes, and urine protein quantification [22-24]. Therefore, it is necessary to follow-up, when the risking factors above are present.

Biopsy is the main criterion for IMN diagnosis. Under a light microscope, mild proliferation of mesangial cells can be seen in the early stage, and the mesangial matrix gradually increases, glomerular sclerosis appears, and diffuse capillary wall thickens in the later stage [25]. The results in this study showed that the proportion of glomerular sclerosis in the elderly group is significantly higher than that in the young and middle-aged group ($P<0.05$). Considering that the membranous nephropathy progresses slowly, it was speculated that the higher proportion of glomerular sclerosis in the elderly group is likely due to its longer course of disease and accompanying diseases such as hypertension, which was consistent with previous reports [24]. Accumulating evidence suggests that severe chronic tubular interstitial damage is a risk factor for progres-

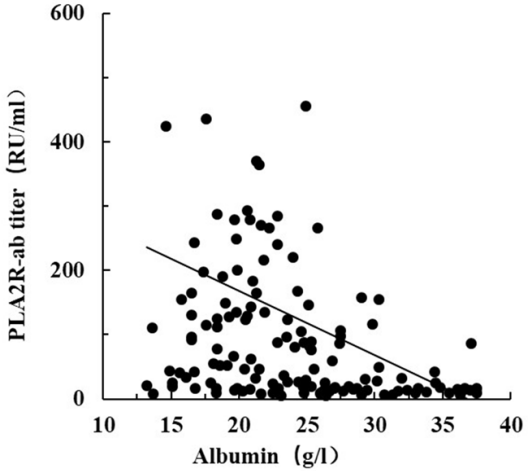

Figure 1. Correlation between PLA2R antibody and albumin in the serum of IMN patients.

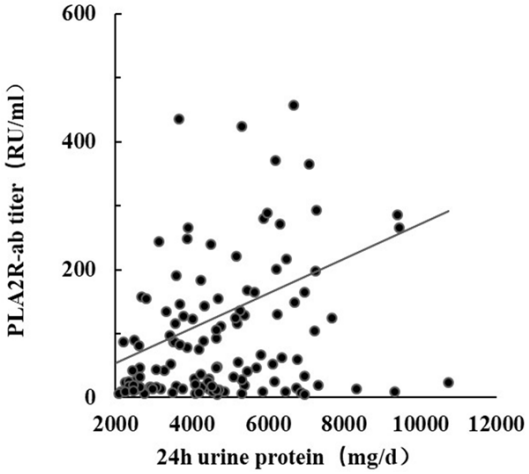

Figure 2. Correlation between PLA2R antibody and 24 h urine protein in the serum of IMN patients.

sion of end-stage renal disease [26]. In this study, the proportions of tubular atrophy, tubular interstitial fibrosis, and inflammatory cell infiltration in elderly group were higher than those of the middle-aged and young group, but the difference was not statistically significant, which requires further evidence by expanding the sample size.

For detection of early deposits, immunofluorescence is usually more sensitive than light microscopy or electron microscopy [27]. It is believed that IgG and C3 in IMN are deposited under the epithelium and basement membrane of the glomerular capillary loop [28]. In addition, it is usually accompanied by renal tubulointerstitial and damage in small blood vessels, which results in thickening of the basement membrane and disappearance of the podocyte foot process [29]. In this study, the immunofluorescence deposition in the glomerulus mainly showed IgG4 (88.03%) and C3 (97.89%), some accompanied by IgM (21.83%) and C1q (36.62%) deposition, and few accompanied by IgA (7.04%) and Fib (5.63%). Deposition of different immune complexes (IgA, IgM, C1q) in multiple locations (mesangial area, subepithelial, basement membrane) were observed in some cases of all three groups. Generally, the possibility of secondary membranous nephropathy should be ruled out when obvious proliferation of mesangial cells, positive immunofluorescence and multi-site deposition of electronic compacts happens, and the secondary factors were also not observed in the patients included in this study. Furthermore, recent studies have indicated that air pollution is highly related to the increased incidence of membranous nephropathy [30], thus the relation between environmental factors and the clinical and pathological aspects of IMN patients should be given more attention.

Anti-PLA2R antibody in serum has good sensitivity and high specificity to IMN, and can be used as a specific biomarker for the diagnosis of IMN. It is reported that anti-PLA2R antibodies in serum was present in about 70% of IMN patients, and this rate likely reached 83% in China [30]. Liu *et al.* [31] compared the expression of PLA2R in frozen and paraffin kidney biopsy specimens and found that frozen specimens were better for diagnosis of IMN. Therefore, frozen kidney biopsy specimens were used in this study to detect PLA2R in glomerular by immunofluorescence staining. Kim *et al.* [32] showed that the PLA2R antibody titer of patients with IMN is positively proportional to urine protein but inversely proportional to renal function and serum albumin. Similarly, Radices *et al.* [33] also revealed that the IMN patients who were positive for PLA2R antibodies displayed more obvious clinical symptoms with greater urine protein and lower serum albumin than those negative for antibodies. However, in our study the serum PLA2R antibody of IMN patients was negatively correlated with serum albumin with correlation coefficient r=-0.269 (P=0.001), and had no correlation with 24 h

urine protein (r=0.113, P=0.180). Moreover, the combined results of serum PLA2R antibody and glomerular PLA2R antigen in IMN patients were as follows: both positive 73 cases (51.41%), both negative 14 cases (9.86%), only serum PLA2R antibody positive 23 cases (16.20%) and only glomerular PLA2R antigen positive 34 cases (23.94%), which was consistent with the findings by Hu *et al.* [34]. Therefore, it is very necessary to track the serum PLA2R antibody dynamically in order to understand its significance.

In this study, eGFR in IMN patients decreased progressively with age (P=0.001), and the degree of glomerulosclerosis, renal tubular atrophy, and renal arteriole wall thickening in the elderly group were significantly higher than those in the young and middle-aged groups (P=0.01, P=0.004, P=0.016), consistent with previous studies that the proportion of glomerular sclerosis, renal tubular atrophy, renal interstitial changes, and small artery disease in patients with IMN increased significantly with the decrease of eGFR [35], while serum anti-PLA2R antibody, serum albumin, and 24-hour urine protein quantification showed no differences among groups.

In summary, the proportion of IMN patients with severe clinical and pathological manifestations was increased in elderly group, i.e. comparing to old patients, young IMN patients have a shorter course of disease, less kidney function damage, and a lower proportion of renal pathological glomerulosclerosis, which indicates that individualized treatment in appropriate timing should be given for patients of different ages and risk levels.

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Disclosure of conflict of interest

None.

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Review Form of Medical Ethics Committee in Shenmu Hospital

No. Research project sm013

Project Name	Analysis of serum PLA2R antibody titers, clinicopathological features and prognostic factors in patients with membranous nephropathy		
Project Source			
Project Department	General Internal Medicine	Project Leader	Xiong Yang
Meeting Location	Conference Room	Meeting Date	December 1, 2020
Eligibility Evaluation for Principal Investigator: **Qualified**			
Evaluation of research proposals: **Compliant with hospital requirements** Date: **December 1, 2020**			
Evaluation of informed consent: **Compliant with hospital requirements** Date: **December 1, 2020**			
Others: **None**			
Voting	Attendance: **18**	Valid votes: **18**	
Audit Result	☑ Agree		
	☐ Agree after necessary modifications		
	☐ Discuss after necessary modifications		
	☐ Disagree		
Recorder's signature: **Chunjiao Jia**		Signature of the Director of the Ethics Committee: **Qiang Wang** (stamp)	

Figure S1. Scanned file of The Institutional Review Board (IRB) Approval Report. This study was approved by the Ethics Committee of Shenmu Hospital Affiliated to Northwest University.

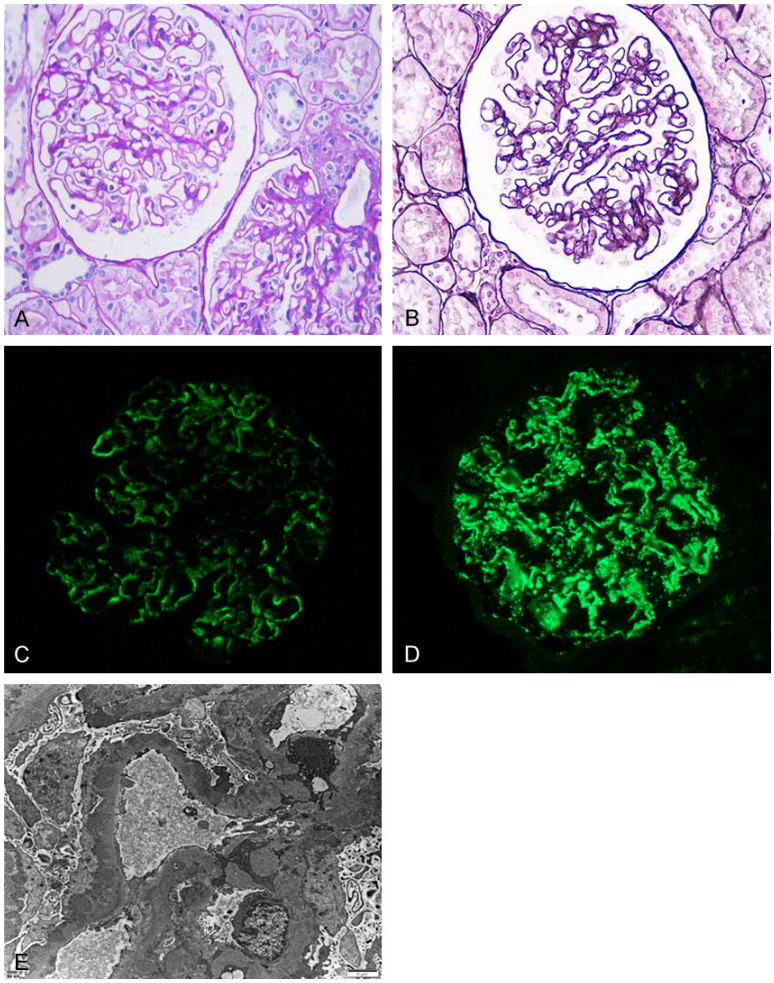

Figure S2. Representative images of renal biopsies showing membranous nephropathy. A. Light microscopy of PAS staining (400 ×); B. Light microscopy of PASM hexamine silver staining (400 ×); C. Immunofluorescence staining of PLA2R; D. Immunofluorescence staining of IgG4; E. Electron micros copy.