Anti-PLA2R and anti-THSD7A as Diagnostic Serological Markers of Idiopathic Membranous Nephropathy: A Single Centre Study

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Abstract

Idiopathic Membranous Nephropathy (IMN) is a renal-limited autoimmune disease and approximately 80% of MNs. The study aimed to evaluate the role of circulation Anti-PLA2R and anti-THSD7A autoantibodies in the diagnosis and differentiation between primary and secondary MN. The study was conducted on 58 adult patients with biopsy- proven MGN. All were subjected to measurement of Anti-PLA2R1 Enzyme-Linked Immunosorbent Assay and anti-THSD7A was detected by indirect immunofluorescence assay. Among the 58 patients, 79.3% were diagnosed as IMN, 20.7% were SMN. Among IMN patients, 32 patients (69.6%) showed positive anti-PLA2R1 antibodies, 2 patients (4.3%) were positive for anti-THSD7A antibodies and the remaining 12 patients (26.1%) were negative for both types of antibodies. Patients with secondary MN were negative for two antibodies. The IMN patients had lower serum creatinine compared to the secondary MN patients (p=0.017). In conclusion, the study demonstrates that serum circulating autoantibodies against PLA2R is a fast, easy, sensitive, and non-invasiveness test for diagnosis of IMN, the combination between serum anti-THSD7A antibodies and anti-PLA2R antibody, may be more sensitive and specific for diagnosis of IMN.

Introduction

Membranous nephropathy (MN), is the most common cause of nephrotic syndrome among adults [1]. The incidence rates of MN are increasing year by year [2]. It is the second or a third important reason of end stage renal diseases in patients with idiopathic glomerulonephritis and are the principal glomerulopathy that relapses after kidney transplantation [3].

MN is a morphological form of damage characterized by an increase in of the glomerular capillary wall thickness due to deposition of immune complexes under the epithelium and complement components with the new basement membrane formation [4].

primary MN(PMN) can be a renal-limited autoimmune disease and approximately 80% of MNs , the residual 20% are secondary (SMN) to different systemic diseases, autoimmune diseases, infections (hepatitis B), malignancy and drugs [5].

Differentiating PMN and SMN depend on their clinical manifestations and laboratory tests, and this is important for diagnosis, treatment and follow-up but it is very difficult [6]. PA kidney biopsy is the gold standard in making the diagnosis, but a biopsy is invasive, costly, and risky [7]. So using less or non-invasive methods; serum samples, urine samples make a diagnosis and follow up patients is very important.

PMN is an organ-specific autoimmune disease, in which circulating autoantibodies bind to an autoantigen on the surface of the podocyte: The M-type phospholipase A2 receptor 1 (PLA2R) and thrombospondin type 1 domain-containing 7A (THSD7A) [4].

PLA2R is an autoantigen of type I transmembrane receptor and one of four mammalian members of the mannose receptor family [8], it is present in glomerular podocytes, its extracellular domain is the transformed antigen which stimulates autoimmune reactions. Attached with the antiPLA2R antibodies formed in the body were mainly IgG4, lead to formation of in situ immune complex which stimulates the complement components causing podocyte damage, with production of protein in urine, that is the key pathogenic feature for PMN patients. [9]

THSD7A is a second autoantigen expressed by the podocyte, [10]. it stimulates IgG4-predominant humoral immune responses that produce circulating autoantibodies that directly affect podocyte integrity, causing damage of cell and proteinuria that can be used clinically for diagnostic and monitoring of adult PMN [11]

Objectives

This work was aimed to evaluate the role of circulating Anti-PLA2R and anti-THSD7A autoantibodies in the diagnosis and differentiation between primary and secondary membranous nephropathy.

Subjects and methods

Patients and samples

The study was conducted on 58 adult patients with biopsy-proven MGN who were attended Nephrology unit Internal Medicine Departments, Benha University Hospital, between January 2018 and April 2019.

Diagnosis of IMN and SMN were based on kidney biopsy and clinical evaluation. Patients with chronic diseases (viral infection B or C, diabetes nephropathy, cancer, lupus nephritis type V was considered to be SMN.

This study was approved by the Research Ethics Committee, Faculty of Medicine, Benha University, Egypt. A written consent from patients was obtained before including in the study.

Serum samples from every patient were collected and immediately aliquoted, frozen and kept at −80°C until tested.

Measurement of Anti-PLA2R1

Anti-PLA2R antibodies were measured using a commercially available ELISA (EUROIMMUN AG, Lübeck, Germany, Order no. EA 1253-9601) that contained PLA2R1-coated microplates. The ELISA was performed according to the manufacturer’s instructions.

patients' serum was diluted in the ratio 1: 101 then incubated in the microplate at room temperature for 30 min. After washing, the microplate was incubated with peroxidase-conjugated anti-human

IgG diluted 1:1,000 in sample buffer at room temperature for 30 min. The color was developed by the addition of chromogen substrate solution for 15 min then stopped by 0.5M H2SO4 stopping solution. Then the optical density was examined at 450 nm (Bio-Rad 550, Tokyo, Japan). All experiments were performed in duplicate and data represents mean values. Antibody positivity was defined as a level over 20 U/mL.[12].

Measurement of Anti-THSD7A immunofluorescence assay

Anti-THSD7A total IgG was detected by indirect immunofluorescence assay kit (EUROIMMUN AG, Lübeck, Germany, Order no. FA 1254-1005-1)) following the standard instructions.

Test principle: THSD7A-expressing cells are incubated with diluted patient samples. If the reaction is positive, specific antibodies attach to the antigens. Then, the attached antibodies are stained with fluorescein-labelled anti-human antibodies and become visible with a fluorescence microscope.

Patients' plasma was diluted 1:10 then 30 µl of diluted sample incubated on the reaction fields of slides at room temperature for 30 minutes. After washing the BIOCHIP slides with a flush of PBS Tween, the slides were incubated with 25 µl of fluorescein labelled anti human globulin to at room temperature for 30 minutes with protection from direct sunlight. Then the slides were examined by fluorescence microscopy (Carl Zeiss AG, Germany).

Statistical analysis

Normally distributed continuous variables were expressed as the mean ± standard deviation (SD) while the median (interquartile) was used for variables that were not normally distributed.

Continuous data were compared using the Student's t-test or the Mann-Whitney test, as appropriate. Categorical data were compared using the Chi2 test. All P values were 2-tailed, with $P<0.05$ was considered statistically significant.

Results

A total of 58 patients with biopsy proven MN were included in the study. The characteristics of these patients are shown in table 1. Most of these patients were males (55.2%). The mean age was (51.9 ± 11.6).

Among the 58 patients, 46 patients (79.3%) were diagnosed as idiopathic membranous nephropathy and 12 patients (20.7%) were secondary MN.

The etiology of secondary MN (n = 12) were lupus membranous nephritis (n = 3), Hepatitis B virus (n=6) and malignancy-associated MN (n = 3).

Comparison between PMN and SMN is shown in table 1. The IMN patients had lower serum creatinine compared to the secondary MN patients (p=0.017). There were no significant differences in age, gender, proportion of hypertension, proteinuria, nor serum albumin between idiopathic and secondary membranous nephropathy patients.

Table (1) Clinical characteristics of primary and secondary MN patient groups

Characteristic	I MN (n=46)	SMN (n=12)	Total (n=58)	P value*
Age (median ± interquartile range)	53 ± 15.75	45.5±20.25	52.5 ± 18	0.40
Gender (male %)	60.9%	33.3%	55.2%	0.91
Hypertension n (%)	26 (56.5%)	5 (41.7%)	53.4%	0.358
Serum Creatinine (mean ± SD)	0.95±0.27	1.17±0.32	0.99±0.29	0.017
Urinary protein (g/24 h) (mean ± SD)	5.22±2.29	5.67 ±2.46	5.31±2.31	0.55
Serum albumin (g/L) (mean ± SD)	27.1 ±6.47	26.32±6.31	26.94±6.39	0.71

IMN – idiopathic membranous nephropathy; SMN – secondary membranous nephropathy

Serological characterization

Sera of all 58 patients were analysed by ELISA for the existence of antibodies against PLA2R1, and by indirect immunofluorescence for the presence of antibodies against THSD7A fig (1).

Among idiopathic MN patients, 32 patients (69.6%) showed positive anti-PLA2R1 antibodies fig (1), 2 patients (4.3%) were positive for anti-THSD7A antibodies and the remaining 12 patients (26.1%) were negative for both types of antibodies. All patients with secondary MN were negative for both antibodies.

Serologic testing for anti-THSD7A antibody using IFT showed that 2 patients only showed positive results, their clinical data are shown in table (2).

According to clinical and serological results, cases can be classified into four groups: PLA2R-Associated (n=32), THSD7A-Associated (n=2) and Double-Negative primary MN (n=12) and secondary MN cases (n=12). We compared the clinical differences between these groups as shown in Table (3). There was no significant difference among groups in gender (p=0.38), age (p=0.157) nor serum creatinine (p=0.121). The variation, however, was statically significant between groups in case of urinary protein (p=0.046), serum albumin (p=0.027) and hypertension (p=0.042).

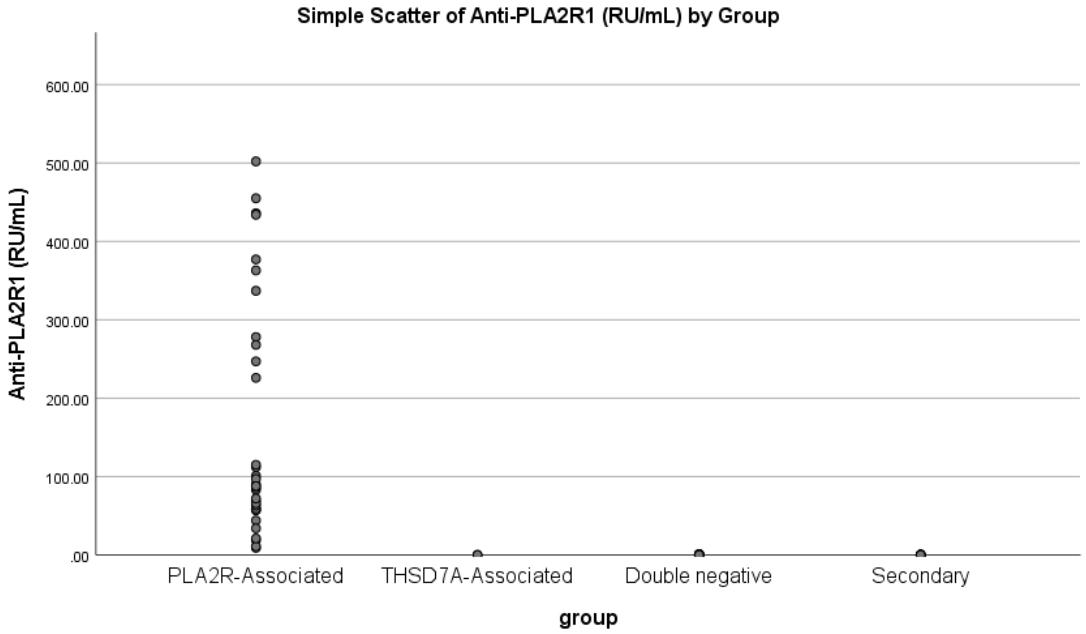

Figure (1) Anti-PLA2R1 levels in PLA2R-Associated MN (n=32), THSD7A-Associated (n=2) and double negative idiopathic MN (n=12) and Secondary MN (n=12)

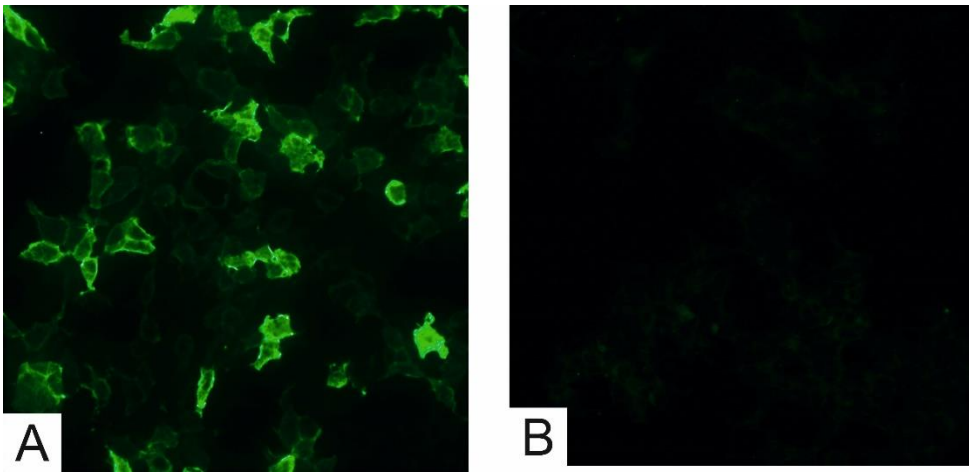

Figure (2): Detection of serum anti-THSD7A antibody by indirect immunofluorescence test. (A) Immunofluorescence pattern of a positive case for anti-THSD7A antibodies. (B) Immunofluorescence pattern of a negative case.

Table (2) The clinical characteristics of THSD7A-Associated IMN patients.

Case No	Age	Gender	Serum anti-THSD7A Ab	Anti PLA2R ab	24 h-proteinuria (g/24h)	Albumin (g/l)	Serum creatinine (mmol/L)	Hypertension
30	47	male	+ve	-ve	3.44	32.51	0.91	+ve
44	53	female	+ve	-ve	6.26	23.9	0.82	+ve

PLA2R – M-type phospholipase A2 receptor; THSD7A – thrombospondin type-I domain-containing 7A.

Table (3) Comparison of clinical data among different serological groups

Characteristic	Idiopathic			Secondary (n=12)	Total (n = 58)	P value
	PLA2R-Associated (n= 32)	THSD7A-Associated (n=2)	Double-Negative (n=12)			
Gender (male %)	62.5%	50%	58.3%	33.3%	55.2%	0.380
Age (median ± interquartile range)	55 ± 17.75	50	50±21.75	45±20.25	52.5 ± 18	0.157
Urinary protein (mean ± SD)	5.82±1.92	4.85±1.99	3.7±2.64	5.67±2.46	5.31±2.31	0.046
Serum albumin (mean ± SD)	25.32±5.21	28.2±6.09	31.67±7.67	26.32±6.31	26.94±6.39	0.027
Serum Creatinine (mean ± SD)	0.95±0.27	0.87±0.06	0.96±0.28	1.17±0.32	0.99±0.29	0.121
Hypertension n (%)	65.6%	100%	25%	41.7%	53.4%	0.042

Discussion

Membranous nephropathy (MN), has paid attention of people in current years for its increasing frequency so the discovery of serum biomarkers may make the diagnosis and treatment be easier [13].

We examined the presence of anti-PLA2R and anti-THSD7A antibodies in Egyptian patients with MN. In our study, among 46 PMN patients, 32 patients (69.6%) showed positive anti-PLA2R1 antibodies.

In 2009, it was discovered that about 70%of patients with PMN have auto-antibodies against the M-type phospholipaseA2 receptor.[13].

A meta-analysis study revealed that the rate of anti-PLA2R in 1ry MN ranged from 57-82%.[14].

Many studies have established that the levels of anti-PLA2R autoantibodies in IMN patients are significantly higher than in SMN patients. [15-17].

Another meta-analysis study reported that, using ELISA, the clinical sensitivity is usually between 72 and 82% when testing is limited to individuals with nephrotic-range proteinuria [18-19]

A study carried out on Chinese population reported that, serum antiPLA2R antibody positive rate was 87.50%. Whereas, this rate for the SMN was 25% and 0.00% for non-MN groups [9].

In our study, neither anti-PLA2R antibodies nor anti-THSD7A was found in secondary MN. In the pioneer study, anti-PLA2R antibodies cannot be detected in 2ry MN**. [20].**

Our results are in agreement with most studies that reported that the majority of their secondary MN patients are PLA2R-negative [12, 22-24].

Other studies have found variable positivity of these antibodies in 2ry MN. It was 9.3% (11 patients) and 2.4% (five patients) for anti-PLA2R and anti-THSD7A antibodies respectively in Chinese study. [21]. Only one out of 35 of secondary MN cases had antipla2r abs in another study. the presence of these antibodies in 2ry MN may present only coexistence of primary MN and other disorder. [25].

A meta-analysis of fifteen studies reported that the anti-PLA2R antibody sensitivity was 78% while its specificity was 99%. In early clinical studies positive rate of anti-PLA2R antibody in IMN patients was between 70 and 80% then decrease in some new studies with different ethnic groups [3].

Some PMN cases may be serum anti-PLA2R antibodies negative due to serum or plasma samples were taken, and the patient was in decrease activity or inactive , all antibodies adsorbed by the kidney (sink theory) or new or cryptic auto- antigens may present, ([8].

PMN that not diagnosed by anti-PLA2R Ab may be diagnosed by, anti-THSD7Aautoantibodies In our study, positive serum anti-THSD7A Ab was detected in two cases of 46 PMN (4.3%)., while a rate of 5%was detected by Seitz-Polski B and his colleagues. [27].

In contrast to our result, auto-antibodies against THSD7A were detected in about 8 – 14% of patients with primary PLA2R-negative MN. [23].

anti-THSD7A Ab may be occurring with malignancy A study was done in 2020 supported that anti-THSD7A Ab was positive in four cases with PMN and two cases of SMN, one of them had a malignancy [28].

In a meta-analysis of ten studies, was reported that the rate of anti-THSD7A was 3% in all patients and 10% between patients with negative anti-PLA2R. and the difference was explained due the difference in sample size or among the races [10].

Regarding to disease activity and Prognosis of MGN with association with anti-PLA2R Ab, Anti-PLA2R levels often mirror disease activity so effective and continues measurement its level and follow up its change provided us with patient immune status in comparing with using proteinuria to evaluate the severity and time and duration of treatment [9].

In our study ,there were statically significant between groups in case of urinary protein and serum albumin .This result is in agreement with many studies that showed that these parameters were higher in the early stage of the disease with severe proteinuria, and then decreased significantly or vanished totally in its remission and slowly increased in the its reappearance [3,9,20].
Serum anti-THSD7A does not show any significant relation with laboratory parameters, e.g. serum creatinine, albumin, and proteinuria levels and this was in agreement with our study [29,30].

CONCLUSION

Measurement of serum circulating autoantibodies against PLA2R has the advantages of rapid, simple, sensitive, and non-invasiveness for diagnosis of PMN, the combination between serum anti-THSD7A antibodies and anti-PLA2R antibody, may be more sensitive and specific for diagnosis of PMN.so a renal biopsy may not always be necessary for diagnosis or treatment decisions . More large and long-term follow-up studies are recommended.

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