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Original Article

The Role of Serum Levels of Anti-Phospholipase A2 Receptor Antibodies in the Diagnosis of Primary Membranous Nephropathy

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Abstract

Introduction: Primary membranous nephropathy (PMN) is one of the most common causes of nephrotic syndrome in adults. The disease process is probably initiated by the binding of circulating autoantibodies to target podocyte antigens. In 2009, Beck et al. found that phospholipase A2 receptor (PLA2R1) was expressed on human podocytes in patients with PMN. Recent evidence suggests that PLA2R1 autoantibodies play an important role in the diagnosis of PMN.

Aim: The aim of the present study was to compare the serum levels of anti-PLA2R1 in patients with PMN, second MN (SMN), other nephropathies (ON), and healthy controls (HC).

Materials and methods: The study included 52 patients with PMN, 12 patients with SMN, 49 patients with ON, and 50 healthy controls. The serum concentration of anti-PLA2R1 was determined with ELISA kit (Anti-PLA2R ELISA, IgG, EUROIMMUN, Lübeck, Germany) using MR-96A microplate Reader (MINDRAY). Statistical analysis was performed with SPSS v.22.0.

Results: There was significant difference in the serum anti-PLA2R1 concentrations between patient groups and HC ($p<0.0001$). Compared to HC, the median anti-PLA2R1 level in the PMN group was significantly higher (4.8 RU/ml vs. 34.9 RU/ml, $p=0.001$), in the ON group it was lower (2.1 RU/ml, $p=0.002$) and did not differ in patients with SMN (2.9 RU/ml, $p=0.193$). The anti-PLA2R1 serum levels were significantly higher in the PMN group than in the SMN ($p=0.015$) and ON ($p<0.001$) groups.

Conclusions: Our results showed that anti-PLA2R1 is significantly increased in patients with PMN. We can conclude that the anti-PLA2R1 serum concentration may be used as a beneficial biomarker for distinguishing PMN from other membranous nephropathies.

Keywords

anti-phospholipase receptor antibodies, glomerulonephritis, membranous nephropathy, secondary membranous nephropathy

INTRODUCTION

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults. Approximately 80% of MNs are PMN, the remaining 20% are secondary to other systemic diseases or due to exposure to other factors including infections, tumours, autoimmune diseases, drugs, etc.[1] These diseases are known as SMN.[2]

Much of the knowledge about the pathogenesis of MN is derived from the observation of experimental models.[3,4] Studies of the Heymann nephritis model of MN in rats in the late 1970s found that the subepithelial immune deposits form in situ when circulating antibodies bind to an intrinsic antigen in the glomerular capillary wall.

In 2009, Beck et al. found that the phospholipase A2 receptor (PLA2R1) was expressed on human podocytes in patients with PMN.[5] Combined with the anti-PLA2R1 antibodies produced in the body, it forms an in situ immune complex that activates the complement system to cause podocyte injury, resulting in urine protein production, which is the major pathogenic factor in the majority of PMN patients.[6] Current clinical studies indicate the significance of circulating autoantibodies against the M-type Phospholipase A2 receptor in the pathogenesis of PMN.[7-10]

A major place in the diagnosis and therapy of MN patients is occupied by the differentiation of primary from secondary forms.[11-13] In this regard, clinical, immunological and histological data are used. The interest toward serum anti-PLA2R1 antibodies has been increasing in the last years.

In the present study, we examined the serum levels of antibodies to the PLA2R1 in different groups of patients with kidney disease. We hypothesized that serum concentration of PLA2R1 in patients with PMN is higher than that in other groups and could improve the differential diagnosis between groups.

AIM

The aim of this study was to compare the serum levels of anti-PLA2R1 in patients with PMN, second MN (SMN), other nephropathies (ON), in and healthy controls (HC).

MATERIALS AND METHODS

One hundred and thirteen patients (73 men and 40 women) who were treated in the Clinic of Nephrology at Kaspela University Hospital, Plovdiv for nephrological diseases were studied. They were divided into three groups: patients with PMN (n=52), patients with SMN (n=12) and patients with ON (n=49). Each patient was diagnosed by a nephrologist. All patients underwent renal puncture biopsy to confirm the diagnosis of MN. The PMN group included patients with histological data on MN and lack of clinical, laboratory, and pathomorphological evidence of diseases that can lead to SMN. In addition, none of the patients had a history of systemic lupus and other autoimmune diseases, tumour processes, chronic infections (HBV, HSV, HIV), medication intake. The SMN group included patients with histological data for MN and clinical, laboratory, and pathomorphological data for diseases that can lead to SMN. The ON group included patients with histological data from a biopsy for other glomerular diseases: minimal change disease (n=9), tubulointerstitial nephritis (n=8), diabetic nephropathy (n=17), hypertensive nephrosclerosis (n=6), light chain nephropathy (n=1), membranoproliferative glomerulonephritis (n=2), focal segmental glomerulosclerosis (n=3), amyloidosis (n=1), antiphospholipid syndrome (n=1), and lupus nephritis (n=1).

The HC group (n=50) was used to compare the data from the pathological groups. The samples of the HC were appropriately selected to match the age and sex distribution of the three patient groups. Clinical and routine hematological, biochemical, and urine tests were performed to assess their health status. Inclusion criteria were voluntary participation, clinical health, routine hematological, biochemical and urinary parameters in the relevant reference intervals. The exclusion criteria included: acute or chronic diseases (inflammation disorders, liver, renal, cardiovascular, endocrine or malignant diseases, immunological disorders), pregnancy and lactation.

Written informed consent for participation in the study was obtained from all patients. The study protocol was approved by the Ethical Committee of Medical University of Plovdiv.

Venous blood samples were collected from all participants in the morning, after an overnight fast, and centrifuged. The serum concentration of anti-PLA2R1 was determined using an ELISA kit (Anti-PLA2R1 ELISA, IgG, EUROIMMUN, Lübeck, Germany) using an MR-96A microplate reader (MINDRAY). The ELISA method is highly specific and sensitive. The method has a sensitivity of 97.5% and a specificity of 100%.[14] Intra- and inter-assay coefficients of variation were less than 5.7% and 10.3%, respectively.

Statistical analysis was performed using SPSS Statistics for Windows, version 24.0 (Armonk, NY: IBM Corp.). Measurement data are presented as n, mean ± standard deviation, median (25th percentile; 75th percentile) or percentage (%) as indicated. The Kolmogorov-Smirnov test was used to analyze the type of distribution of the results. Non-parametric Kruskal-Wallis and Mann-Whitney U tests were used to compare the anti-PLA2R1 data between groups. A P value of <0.05 was considered to be statistically significant.

RESULTS

Table 1 presents data on the age and sex distribution of persons from all four groups included in the study. There were no differences between the groups with respect to age (p=0.055) and sex (p=0.872). In all groups, the relative share of men was higher than that of women (1.6:1).

After checking for normal distribution, Kruskal-Wallis test was used to compare the anti-PLA2R1 results. **Table 2** shows that median serum anti-PLA2R1 levels differ significantly between groups (p<0.0001).

Then a pair-wise comparative analysis was performed between PMN and HC, SMN and HC, ON and HC with

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Table 1. Demographic data of patients with PMN, SMN, ON and HC

Characteristic		Groups			
		PMN n=52	SMN n=12	ON n=49	HC n=50
Age (years)		52.6±13.8	57.3±16.1	58.7±12.4	53.1±8.2
Gender, n (%)	Men	32 (61.5)	8 (66.7)	33 (64.6)	30 (60)
	Women	20 (38.5)	4 (33.3)	16 (32.7)	20 (40)

Table 2. Comparative data for anti-PLA2R1 (RU/ml) in patients with PMN, SMN, ON and HC

Groups	Median	Min - Max	25th pct – 75th pct	Kruskal-Wallis *p*
PMN	34.9	0.5-3859.0	2.7-122.8	<0.001
SMN	2.9	0.8-8.1	2.0-3.6	
ON	2.1	0.2-16.8	1.2-3.6	
HC	4.8	0.2-19.8	3.8-10.7	

the Mann-Whitney U test. Compared to HC (4.8 RU/ml) the median value of anti-PLA2R1 in the PMN group (34.9 RU/ml) was significantly higher (p=0.001) and in ON group (2.1 RU/ml) was significantly lower (p=0.002). Anti-PLA2R1 levels did not differ between SMN (2.9 RU/ml) and HC (p=0.193).

Comparisons of anti-PLA2R1 levels between patient groups showed significantly higher levels in PMN group than those in SMN (p=0.015) and ON (p<0.001) groups. There was no statistically significant difference between patients with SMN and ON (p=0.191).

DISCUSSION

MN is caused by an array of conditions with different etiologies and pathogeneses.[15] MN is an autoimmune disease usually associated with a nephrotic syndrome and it is a unique glomerular lesion.[2,16] About 80% of cases are PMN and 20% are associated with other systemic diseases or exposures and are called SMN.[2] The M-type PLA2R has been identified as the probable main target antigen.[17] Although the pathogenesis of idiopathic MN remains incompletely defined, an important advance has been obtained by the seminal study of Beck et al.[5], who showed that approximately 70% of patients with PMN had circulating autoantibodies directed against PLA2R1 located on the surface of normal human podocytes. Autoantibodies to PLA2R1, usually of the IgG4 subclass, can bind to conformational epitopes on specific domains of PLA2R1 expressed on the podocyte surface.[16]

Determination of anti-PLA2R1 antibody levels in serum is a new parameter that is not yet widely implemented in laboratory practice. A review of more than 230 research publications on MH revealed that only a small number of them were dedicated to this parameter. For example, Wu X et al. reported increased anti-PLA2R1 antibody levels in PMN patients in comparison with patients with SMN and ON using the same ELISA method.[6] We also found that serum levels of anti-PLA2P1 were statistically higher in patients with PMN compared to other patient groups. Our data, as well as those of Wu X et al., indicate that serum levels of anti-PLA2R antibodies can be used to distinguish PMNs from SMNs and ON, which also determines their significance.

In our study, there was statistically significant difference in the median values for anti-PLA2R1 concentrations between patient groups and HC (p<0.0001). Compared to controls, the median serum anti-PLA2R1 value of PMN group was significantly higher (4.8 RU/ml vs. 34.9 RU/ml, p=0.001). Liu Y et al. also found that the median serum anti-PLA2R1 value of PMN group was significantly higher than that of the HC group (36.6 RU/ml vs. 1.7 RU/ml, p<0.05).[18]

We have not established statistically significant difference in anti-PLA2R1 between SMN patients and HC (p=0.193). ON patients showed lower anti-PLA2R1 than the healthy controls (p=0.002). The analysis of our data showed that serum levels of anti-PLA2P1 were statistically higher in patients with PMN compared to other patient groups and HC. A number of scientific publications have similar data to support our findings.[6,18-22]

In the PMN group we identified a woman with HBV and a man with a bladder tumour, both cases with high concentrations of anti-PLA2R antibodies (59.8 RU/ml, 802.27 RU/ml, respectively). It cannot be clearly stated whether these are cases of SMN or PMN in the presence of another disease.

Other studies indicate that measurements of anti-PLA2R levels in the follow-up of patients with PMN allows the serial monitoring of immunologic and clinical activity in these patients. Compared with the traditional approach of

using proteinuria to determine disease severity and treatment efficacy, anti-PLA2R antibody is more effective and agile for monitoring the condition, predicting treatment efficacy, and determining the treatment time and duration.[7]

Clinically, in patients with contraindications for renal biopsy and patients who refuse to undergo renal biopsy, serum anti-PLA2R antibody measurement is feasible. Patients with negative serum anti-PLA2R antibodies and diagnosis of MN must be actively investigated and followed for SMN.[23,24]

Xi X et al.[8] also considered that serum anti-PLA2R antibody levels are significantly related to the condition of MN, and positive expression of anti-PLA2R antibodies is highly consistent with renal biopsy results. The dynamic monitoring of quantitative changes in serum anti-PLA2R antibodies can be used as an important reference indicator to reflect the immune status of PMN patients in real time. Disease monitoring and efficacy of treatment could be used to determine the duration of immunosuppressive therapy.[9] In addition, there is a study showing that serum anti-PLA2R antibody test results before renal transplantation in patients with MN are helpful to predict the risk of recurrence after renal transplantation.[25]

However, there are still many unsolved problems, such as the role of PLA2R and its antibodies in the pathogenesis of PMN. The evaluation of serum anti-PLA2R antibody absolute levels and anti-PLA2R antibody quantification trends and determining which has more clinical significance related to the prognosis of PMN still needs further study.

CONCLUSIONS

Our results showed that circulating anti-PLA2R1 concentration is significantly increased in PMN patients as compared to healthy control and to other forms of MN. We can conclude that determination of anti-PLA2R1 serum concentration is useful for the differentiation between PMN and other MN.

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Роль сывороточных уровней антител к рецепторам фосфолипазы А2 в диагностике первичной мембранной нефропатии

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Резюме

Введение: Первичная мембранозная нефропатия (ПМН) – одна из наиболее частых причин нефротического синдрома у взрослых. Процесс заболевания, скорее всего, начинается со связывания циркулирующих антител с антигенами подоцитов-мишеней. В 2009 году Beck et al. обнаружили, что рецептор фосфолипазы А2 (PLA2R1) экспрессируется на подоцитах человека у пациентов с ПМН. Последние данные свидетельствуют о том, что антитела к PLA2R1 играют важную роль в диагностике ПМН.

Цель: Целью настоящего исследования было сравнить сывороточные уровни анти-PLA2R1 у пациентов с ПМН, вторичным МН (ВМН), другими нефропатиями (ДН) и здоровыми людьми из контрольной группы (ЗК).

Материалы и методы: В исследование были включены 52 пациента с ПМН, 12 пациентов с ВМН, 49 пациентов с ДН и 50 здоровых людей из контрольной группы. Концентрацию анти-PLA2R1 в сыворотке измеряли с помощью ELISA (Anti-PLA2R ELISA, IgG, EUROIMMUN, Любек, Германия) с использованием считывающего устройства для микропланшетов MR-96A (MINDRAY). Статистический анализ проводился с помощью SPSS v.22.0.

Результаты: Наблюдалась статистическая разница в сывороточных концентрациях анти-PLA2R1 между группами пациентов и ЗК (p<0.0001). По сравнению с ЗК средний уровень анти-PLA2R1 в группе пациентов с ПМН был достоверно выше (4.8 RU/ml против 34.9 RU/ml, p=0.001), в группе с ДН он был ниже (2.1 RU/ml, p=0.002) и не было различий у пациентов с ВМН (2.9 RU/ml, p=0.193). Сывороточные уровни анти-PLA2R1 были значительно выше в группе ПМН по сравнению с группой ВМН (p=0.015) и группой ДН (p<0.001).

Заключение: Наши результаты показали, что уровень анти-PLA2R1 был значительно повышен у пациентов с ПМН. Мы можем сделать вывод, что сывороточные концентрации анти-PLA2R1 могут быть использованы в качестве полезного биомаркера для отличия ПМН от других мембранных нефропатий.

Ключевые слова

антитела к рецепторам фосфолипазы, гломерулонефрит, мембранозная нефропатия, вторичная мембранозная нефропатия