SERUM LEVELS OF ANTI-PHOSPHOLIPASE A2 RECEPTOR ANTIBODIES IN PATIENTS WITH MEMBRANOUS NEPHROPATHY

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Abstract: Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in adults [1, 2]. Most patients present with a nephrotic syndrome: proteinuria above 3.0 g/24 h, hypoalbuminemia, edema, hyperlipidemia and lipiduria, and normal, or slightly altered, kidney function. Two forms of MN have been described – the primary form (PMN) and the secondary form (SMN), which represent 70% and 30% of cases, respectively [3, 4 ,5, 6]. The characteristic pathological change is capillary walls thickening resulting from immune complex formation on the subepithelium of the glomerular basement membrane [7].
Most idiopathic MN (IMN) is now believed to be an autoimmune disease where the podocyte is the target and the source of autoantigen [8, 9, 10, 11], such as M-type transmembrane phospholipase A2 receptor or thrombospondin type-1 domain-containing 7A [12, 13]. The remaining patients that are anti-PLA2R negative have antibodies against thrombospondin type-1 domain-containing 7A (THSD7A) [1]. Both autoantibodies have been proposed as biomarkers of MN autoimmune activity [14, 15, 16].
The THSD7A is the most extensively characterized member of a family of extracellular matrix glycoproteins involved in the regulation of cellular behavior during tissue genesis and repair [22]. It interacts with glycosaminoglycans, calreticulin, and integrins regulating cellular adhesion in the extracellular environment. THSD7A mediates interaction of low-density lipoprotein receptor during its uptake and clearance at the surface of various cells and also regulates the interaction with fibrinogen during platelet aggregation.Under normal conditions in humans THSD7A expression is limited to the slit diaphragm, the podocyte's soma, endocytic compartment, and foot processes. THSD7A is found in rodents, but not on the GBM or on endothelial cells [23]. It has been reported that 10% patients with PLA2R1-negative idiopathic MN had detectable anti-THSD7A autoantibodies. As for anti-PLA2R1 antibodies, the circulating serum anti-THSD7A antibodies were mainly IgG4 subtype but a weak presence of other subtypes has also been found [21].
High anti-PLA2R1 antibody levels have recently been reported as a reliable prognostic factor [17, 18, 19, 20] which is likely to modify the indications for treatment to improve long-term outcomes of MN in the future.
Current clinical studies indicate the significance of circulating autoantibodies against the M-type Phospholipase A2 receptor (aPLA2R-ab) in the pathogenesis of PMN. The aim of this study was to compare the level of aPLA2R antibodies between patients with primary membranous nephropathy (PMN), patients with secondary membranous nephropathy (SMN) and healthy controls.
Keywords: membranous nephropathy, phospholipase A2 receptor, antibodies, thrombospondin type-1 domain-containing 7A, nephrotic syndrome

INTRODUCTION

The new pathophysiological concept of alloimmune MN has stimulated research aiming to identify human podocyte antigens that may act as targets for circulating pathogenic antibodies in humans, thus leading the way to the identification in 2009 of the first antigen responsible for primary MN in adults [12].This publicationwas a cornerstone in the history of MN as it provided evidence that iMN in adults is actually an autoimmune disease associated with the production of anti-PLA2R1 antibodies and introduced a new terminology of PLA2R1 related MN [19]. Antibodies against PLA2R1 are of particular clinical importance as they are detected in approximately 70% to 80% of adult cases of MN without apparent secondary causes, particularly in men [12**,** 24]. Circulating anti-PLA2R1 antibodies reflect immunological activity of disease [12] and have been shown to disappear before clinical remission of nephrotic syndrome [12] and to reappear in the circulation before clinical relapse [25].

EXPOSITION

The aim of this study was to compare the level of aPLA2R antibodies between patients with PMN, patients with SMN and healthy controls. The study included 33 patients with PMN (mean age 53.36±14.10 years), 10 patients

with SMN (mean age 55.40±17.54 years) and 35 healthy controls (mean age 45.71±13.07 years). Serum PLA2R antibodies levels were measured with ELISA kit (Anti-PLA2R ELISA, Ig G, EUROIMMUN, Lübeck, Germany). The data was analyzed using SPSS, Version 24. All data was expressed as mean ±SD. Significance was considered at P?0.5.

The data was analyzed with the Statistical Package for the Social Sciences (SPSS), Version 24. The descriptive statistics included means and standard deviations for the continuous variables (age and aPLA2-Ab) and proportions of male and female participants. One-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare the groups in mean age. Sex distribution was examined through crosstabulation and the chi-square test. The aPLA2-Ab distributions were checked for normality with the Kolmogorov-Smirnov test which showed violations in the PMN and SMN groups ($p < 0.01$). Hence, the Kruskal-Wallis nonparametric test was used to compare the three groups, followed by Mann-Whitney pair-wise comparisons. Statistical significance was considered at Type I error *alpha* ? 0.5.

The sample consisted of 78 participants, including 33 patients with PMN (mean age 53.36±14.10); 10 patients with SMN (mean age 55.40±17.54); and 35 healthy controls (45.71±13.07). The mean age of the PMN and SMN patients was similar and not significantly different ($p = 0.92$). The healthy controls were younger than both patients' groups ($p = 0.43$). In the whole sample and each of the three groups, the male patients were predominant (60.3% males in the sample; 66.7% males in the PMN group; 70% males in the SMN group; and 51.4% males in the controls). The proportional distribution of male and female participants in the three groups was not statistically significant, $p = 0.35$. The demographic data is summarized in Table 1.

Table 1: *Demographic data*

Variables		Total (N = 78)	PMN patients (N = 33)	SMN patients (N = 10)	Healthy controls (N = 35)
Age: Mean (±SD)		50.19 (±14.52)	53.36 (±14.10)	55.40 (±17.54)	45.71 (±13.07)
Sex: N (%)	Male	47 (60.3%)	22 (66.7%)	7 (70%)	18 (51.4%)
	Female	31 (39.7%)	11 (33.3%)	3 (30%)	17 (48.6%)

* Significant at alpha ≤ 0.05; ** Significant at alpha ≤ 0.01

The descriptive statistics for aPLA2-Ab and the statistics of the Kruskal-Wallis test are given in Table 2. The results show a statistically significant difference between the groups, $\chi 2 = 60.69$, $p < 0.001$.

Table 2: *Results of the Kruskal-Wallis comparison of the three groups*

Group	N	aPLA2-Ab		Kruskal-Wallis			
		Mean	SD	Mean Rank	χ 2	df	p
PMN	33	309.18	678.70	62.00	6		
SMN	10	2.99	2.0	9.90	0.69	2	.000**
Healthy Controls	35	8.41	4.67	26.74			

* Significant at alpha ≤ 0.05; ** Significant at alpha ≤ 0.01

The pairwise comparisons through the Mann-Whitney test showed that the PMN patients had significantly higher levels of aPLA2-Ab (Mean 309.18) than the SMN patients (Mean 2.99), $p < 0.01$. The PMN patients also showed significantly higher aPLA2-Ab levels than the healthy controls (Mean 8.41), $p < 0.01$. The SMN patients and the healthy controls differed significantly ($p < 0.01$) as higher values of aPLA2-Ab were observed in the healthy controls.

Table 3: *Mann-Whitney pairwise comparisons between the groups*

Pairwise Comparisons	aPLA2-Ab		
	Mean Rank	Sum of Ranks	U
PMN patients	27.00	891.00	
SMN patients	5.50	55.00	0.00
PMN patients	52.00	1716.00	
Healthy controls	18.00	630.00	0.00
SMN patients	9.90	99.00	
Healthy controls	26.74	936.00	44.00

* Significant at alpha ≤ 0.05; ** Significant at alpha ≤ 0.01

The group mean values of aPLA2-Ab are illustrated on Figure 1 in comparison to the reference value of 20, which marks the cut-off point between negative and positive cases. The PMN patients exceeded this value 15 times, whereas the aPLA2-Ab levels in the SMN patients and healthy controls were below the cut-off point.

RESULTS

The PMN patients had significantly higher mean level of aPLA2-Ab compared to SMN patients (309.18±678.70 RU/ml vs 2.99±2.00 RU/ml, $P < 0.01$) and to healthy controls (309.18±678.70 RU/ml vs 8.41±4.67 RU/ml, $P < 0.01$). The SMN patients and the healthy controls differed significantly ($P < 0.01$) as higher mean value of aPLA2-Ab were observed in the healthy controls.

CONCLUSIONS

In PMN patients the higher levels of aPLA2-Ab might be regarded as a new opportunity to more accurately define the etiology of MN and might be used as an indication for close monitoring of such patients.

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