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Original article

Comparing the Level of Immunoglobulins in Patients with Primary Membranous Nephropathy under Standard Treatment and Rituximab

Banafsheh Mosharmovahed[1], Bahareh Mohebbi[2], Saeed Barzegari[3], Katayoun Hasanzadeh[4], Fatemeh Pour-Reza-Gholi[4], Sohrab Ali Ghorbanian[5] and Sara Assadiasl[2]

[1]Department of chemical engineering-pharmaceutical engineering, University of Tehran, Tehran, [2]Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, [3]Department of health information technology, Amol faculty of paramedical sciences, Mazandaran University of medical sciences, Sari, [4]Department of Nephrology, Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, [5]Department of Chemical Engineering, Faculty of Engineering, University of Tehran, Tehran, Iran

Abstract

Introduction. Primary membranous nephropathy (PMN) is an autoimmune disease affecting renal glomeruli, characterized by aggregation of autoantibodies on podocytes and subsequent epithelial thickening. Rituximab (RTX), an anti-CD20 monoclonal antibody, is used to treat steroid-resistant PMN cases. Accordingly, presented study compared peripheral blood IgA, IgM, and IgG as well as IgG subclasses levels in patients under standard treatment and under RTX treatment before and after RTX administration.

Method. With method of nephelometery, 25 PMN patients, 15 under standard treatment and 10 receiving standard+RTX treatment were enrolled and compared with 15 healthy matched individuals. The RTX group was studied before (pre-RTX) and 2 months after (post-RTX) drug infusion.

Results. Serum IgG level of Std-treatment patients and healthy controls was higher compared to pre-RTX group (0.001 and 0.008 respectively). Serum total IgA and IgM levels were not statistically different between Std-treatment, pre-RTX, and healthy controls; however, IgA decreased after administration of rituximab (P= 0.015). IgG1 and IgG2 levels in pre-RTX group was considerably lower than in patients on Std-treatment (P=0.001 and 0.005, respectively) and healthy controls (P=0.041 and 0.019, respectively) but were not affected by RTX. Serum IgG4 was elevated in both Std-treatment and pre-RTX groups compared to healthy controls (P<0.0001 and 0.010, respectively) and decreased significantly following RTX administration.

Conclusion. Serum IgG4 has a significant prognostic value in PMN. IgG1 and IgG2 were lower in steroid-resistant cases. IgM and IgA do not appear to be involved in pathogenesis of PMN. Moreover, IgG1, IgG2 and IgM amounts were not affected by RTX.

Keywords: glomerulonephritis, membranous nephropathy, Immunoglobulin, Rituximab

Introduction

Primary (or idiopathic) membranous nephropathy (PMN) is considered the most common cause of nephrotic syndrome (NS) in adults, characterized by proteinuria, edema, hypoalbuminemia, hyperlipidemia and usually detectable circulating autoantibodies [1,2]. Histopathological features of PMN consist of subepithelial immune complex deposition and subsequent thickening of glomerular basement membrane; in fact, diagnosis of primary membranous nephropathy could merely be confirmed by ruling out secondary causes such as autoimmunity (e.g. lupus erythematous), chronic infections (e.g. HBV, HCV, H.Pylori), drugs (e.g. gold, penicillamine) and malignancies (e.g. renal, prostate), situations which are accompanied by excessive antibody production [3,4]. Although the underlying causes of PMN have not yet been completely discovered, recent studies have introduced some autoantigens on podocytes which are targeted by autoantibodies detectable in blood circulation of most PMN patients [5]. The most known autoantigen herein is phospholipase A2 receptor (PLA2R), a member of the mannose receptor family. The specificity of anti-PLA2R antibody for diagnosis of PMN has been estimated at 70-80% whereas it is undetectable in healthy individuals. Evaluation of anti-PLA2R antibodies is performed for both diagnosis and follow up purposes in clinical field as its presence and persistence is generally accompanied by worse prog-

Correspondence to: Sara Assadiasl, Molecular immunology research center, Children's Medical Center, 62 Qarib St., Keshavarz Blvd., Tehran 14194, Iran; Phone: 00989128238290; E-mail assadiasl@sina.tums.ac.ir

nosis [6]. The other autoantigen targeted by autoantibodies in PMN is thrombospondin type1 domain–containing 7A (THSD7A), membrane-associated N-glycoprotein which mediates endothelial cell migration and tube formation, found in almost 5% of PMN cases [2]. Lecithin-cholesterol acetyl transferase (LCAT), an enzyme involved in maintaining cholesterol homeostasis, has recently been suggested as another probable target [7]. Of note, in contrast to the secondary membranous nephropathies which could be accompanied by increased level of IgG1, IgG2, and IgG3, the prominent autoantibody isotype directed against abovementioned antigens in PMN is IgG4 [4].

In addition to the supportive care including blood pressure control, angiotensin blockers to minimize proteinuria, statins for treating hyperlipidemia, low protein diet, salt restriction and diuretics to control edema, patients with progressive decrease of glomerular filtration rate (GFR) or refractory proteinuria should undergo immunosuppressive therapy by drugs such as steroids, cyclophosphamide, calcineurin inhibitors (cyclosporine, tacrolimus), and finally rituximab (RTX) [8]. RTX has been shown to ameliorate refractory form of PMN demonstrated by reducing urine proteins following administration [9]. RTX a chimeric monoclonal anti-CD20 antibody eliminates autoantibody-producing B cells by direct signaling, complement-mediated cytotoxicity (CMC), and antibody-dependent cellular cytotoxicity (ADCC) [10]. It might also bind directly to the sphingomyelin-phosphodiesterase-acid-like-3b (SMPDL-3b) on podocytes protecting these cells from their disruption [11,12]. Regarding the considerable role of antibodies in pathogenesis of PMN, we aimed to evaluate the level of various antibody subtypes and subclasses in steroid-responsive and refractory PMN patients (before and after receiving RTX) in order to study their predictive and pathologic value.

Material and methods

Patients

Patients were enrolled according to the clinical, laboratory and pathological diagnostic criteria of primary membranous nephropathy, including those who were under standard treatment (steroid and immunosuppersive drugs, shown in table 1) and refractory cases who received standard treatment plus rituximab [13]; the latter group was studied just before administrating rituximab (Pre-RTX) and two months afterwards (Post-RTX).

Table 1. Demographic and laboratory data of studied population (at sampling time)

Group	Healthy control	Post-Rituximab	Pre-Rituximab	Standard treatment	P value
N	15	10	10	15	
Age in years: mean ± SEM	45.8±3.5	52.2±3.49	52.2±3.49	48±3.26	**0.56**
Gender ratio (M:F)	8: 2	8: 2	8: 2	11: 4	
Creatinine: Mean ± SEM	1±0.03	1.44±0.1	1.81±0.1	1.25±0.03	**<0.0001**
BUN: Mean ± SEM	19.2±0.93	36.8±1.36	50.4±2.26	36.9±1.74	**<0.0001**
WBC: Mean ± SEM	5020±270	4342±341	6890±261	6620±261	**<0.0001**
Total lymphocyte percent	42.3±1.47	33.5±1.5	54.1±1.98	48±2	**<0.0001**
Urine protein (mg/dl)	0: 15	<300: 2 <3000: 8	3000-4000: 5 4000-5000: 3 >5000: 2	500-800: 12 1000-1500: 1 1500-3000: 1 >3000:1	
Anti- PLA2R	-	Positive: 1 Negative: 9	Positive: 3 Negative: 7	Negative: 15	
IS protocol:					
Pred, Cyclosporin Pred,	-	7	7	12	
Cyclosporin, Mycophenolate		0	0	1	
Pred, Tacrolimus		2	2	2	
Pred, Mycophenolate, Tac		1	1	0	

Standard treatment: PMN patients under standard treatment (steroid + immunosuppressive); Pre-Rituximab: PMN patients under standard treatment + Rituximab before receiving the drug; Post-Rituximab: PMN patients under standard treatment + Rituximab two months after administration; WBC: white blood cells; IS: immunosuppression; PLA2R: phospholipase A2 receptor; Pred: prednisone; SEM: standard error of mean

Serum isolation and measurement of immunoglobulins

Serum samples were collected in serum separating tubes and were separated immediately using centrifuge. Then they were aliquoted and stored in freezers at minus 70 degrees until the end of sampling period. Immunoglobulins level were measured using nephelometry technique, (MininephTM Human Ig Kit, The Binding Site Ltd., Birmingham, UK).

Statistical analysis

Data were presented as mean ± SEM (standard error of the mean). The comparison between groups was perfor-

med by non-parametric Kruskal-wallis test. Comparison between pre and post RTX values was performed by non-parametric Wilcoxon test (SPSS 22; SPSS Inc., Chicago,USA). P values less than 0.05 were considered as statistically significant.

Results

The demographic and laboratory data of patients and healthy controls are summarized in table 1.

Antibody subtypes difference between groups

Serum levels of IgG, IgA and IgM as well as IgG subcla sses IgG1, 2 and 4 are shown on Table 2. Serum total IgG level in standard-treatment patients were higher than in the pre-RTX group (0.001) but almost similar to healthy controls. IgG levels of pre-RTX patients were significantly lower than in healthy controls (p=0.008) and decreased further after RTX administration (p=0.035) (Figure 1).

Table 2. Immunoglobulins levels in studied groups (mg/dl)

Group	IgG (mean+SEM)	IgA (mean+SEM)	IgM (mean+SEM)	IgG1 (mean+SEM)	IgG2 (mean+SEM)	IgG4 (mean+SEM)
Std-treatment	1167±60	180±4	141±11	615±44	417±24	53±5
Pre RTX	812±60	175±14	130±14	381±38	279±28	40±4
post RTX	638±50	122±13	123±10	344±33	198±32	25±5
Healthy controls	1038±50	152±17	125±9	483±36	389±20	24±2

Std: standard; RTX: Rituximab; SEM: standard error of mean

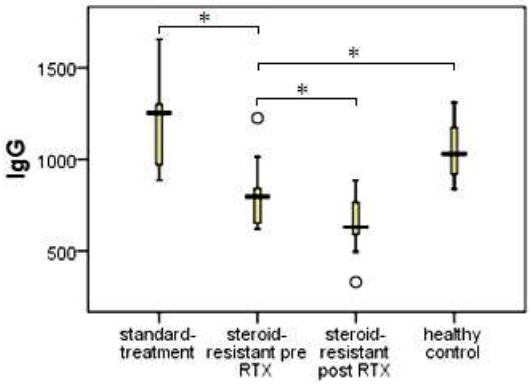

Fig. 1. Serum IgG levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

Serum total IgA levels were not statistically different between Std-treatment, pre-RTX, and healthy control groups; however, they were reduced after administration of RTX (P= 0.015) (Figure 2). Serum total IgM levels were almost similar in all studied groups and were not affected by RTX therapy (Figure 3).

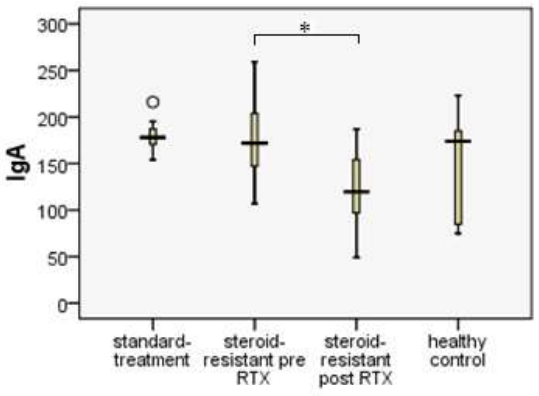

Fig. 2. Serum IgA level in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

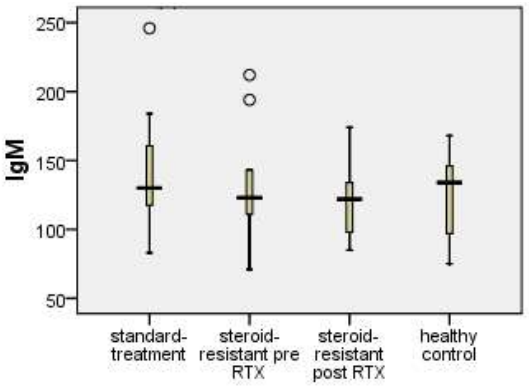

Fig. 3. Serum IgM level in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups

IgG subclasses differences between groups

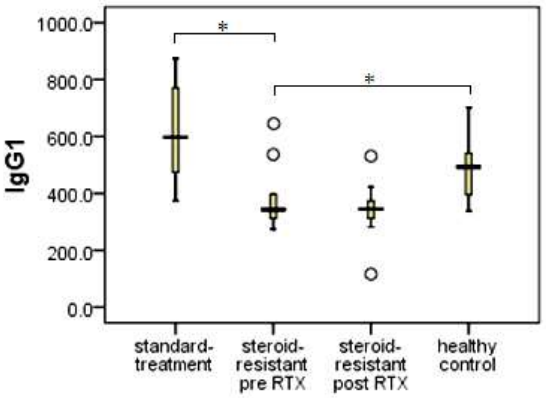

Fig. 4. Serum IgG1 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

IgG1 levels pre-RTX were considerably lower than in patients on Std-treatment (P=0.001) and healthy controls (P=0.041). Moreover, no change was observed after RTX therapy (Figure 4). Similarly, serum IgG2 levels showed significant decrease in pre-RTX group compared to the Std-treatment (p=0.005) and healthy controls (p=0.019) but they were not affected by RTX (Figure 5). Serum IgG4 levels were elevated in both Std-treatment and pre-RTX groups compared to the healthy controls (P <0.0001 and 0.01, respectively) and decreased significantly after RTX administration (P= 0.011) (Figure 6). Comparison of immunoglobulin values is summarized in Table 3.

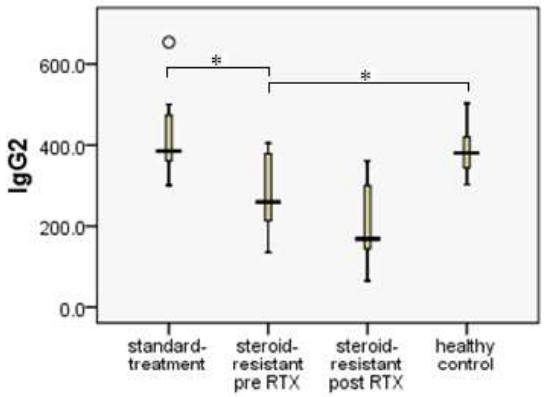

Fig. 5. Serum IgG2 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy groups (*:P.value<0.05)

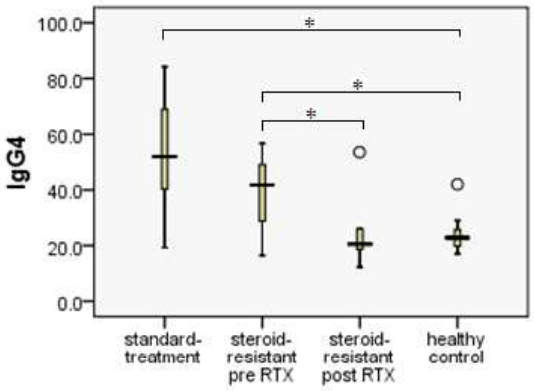

Fig. 6. Serum IgG4 levels in standard-treatment, steroid-resistant (before and after RTX therapy) and healthy control groups (*:P.value<0.05)

Table 3. Comparison of immunoglobulins levels between studied groups

Compared Groups	IgG (p.value)	IgA (p.value)	IgM (p.value)	IgG1 (p.value)	IgG2 (p.value)	IgG4 (p.value)
Std-treatment/pre Rtx	0.001*	0.598	0.523	0.001*	0.005*	0.56
Std-treatment/healthy control	0.202	0.36	0.405	0.067	0.618	<0.000*
Pre RTX /healthy control	0.008*	0.496	0.853	0.041*	0.019*	0.010*
Pre RTX /post RTX	0.035*	0.015*	0.796	1	0.063	0.011*

Std: standard; RTX: Rituximab

Discussion

Recent studies have shown that in most patients with primary membranous nephropathy anti-PLA2R IgG4 auto-antibodies are detectable in blood circulation whereas this is absent in secondary forms of the disease [4]. Although the significance of autoantibodies in pathogenesis of membranous nephropathy has been shown, the probable relationship between antibody level and disease stage or prognosis has not yet been clarified. Bazzi *et al.* assessed the fractional excretion of IgG (FE-IgG) in 84 patients with idiopathic membranous nephropathy and after a follow up period of 7 years they found that higher FE-IgG amounts could predict increased risk of kidney failure and lower possibility of remission. In addition, combined treatment by steroids and cyclophosphamide decreased the progression rate and increased the remission rate in patients with high FE-IgG [14]. In another study Lönnbro-Widgren compared glomerular IgG sub classes deposition between patients with PMN and those with secondary membranous nephropathy (MN) due to malignancy. There was a significant correlation between the absentce of IgG4 and malignancy-related MN while the other IgG subclasses' amount did not differ significantly between the two groups; IgG2-positivity was present in most patients in both groups but neither IgG1 nor IgG3 seemed to be implicated in the pathogenesis of membranous nephropathies [15]. Later, von Haxthausen *et al.* found no difference in level of IgG subclasses between primary and malignancy-associated MN. Additionally, higher level of these antibodies did not show any association with disease progression rate [16]. However, Na *et al.* comparing IgG subclasses in membranous lupus nephritis and idiopathic membranous nephropathy found differences between the two groups apart from IgG1 [17]. Another study revealed IgG4 and IgG1 dominancy in primary and secondary membranous nephropathies respectively. Moreover, at early stages of PMN IgG1 was the most frequent subtype in tissue biopsy although by disease progression IgG4 got the first place [18]. Kuroki *et al.* has evaluated the level of glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis (DPLN), membranous LN lupus nephritis (MLN) and primary membranous nephropathy. They found IgG1, IgG2, IgG3, and at a lesser amount IgG4 in glomerular deposits of DPLN and MLN while IgG4 was the dominant glomerular IgG subclass in PMN.

The mean serum IgG subclasses concentration in DPLN and MLN were almost similar to the healthy controls, except for IgG1 which was increased in MLN. In PMN patients, the mean IgG4 was increased; however, the mean concentration of other IgG subclasses was significantly decreased [19]. Liu *et al.* showed elevated expression of anti-PLA2R antibodies and IgG4 level in PMN patients comparing to the secondary MN as well as non-MN cases. Moreover, a strong correlation was found between anti-PLA2R serum level and tissue deposition [20]. These findings were confirmed by Yeo *et al.* who calculated 83% sensitivity and 88% specificity for PLA2R, and 76% sensitivity and 86% specificity for IgG4 in diagnosis of PMN. When both were positive, the specificity raised up to the 96.4% [21].

Juozapaite *et al.* evaluated the significance of the IgM deposits in the mesangium of children with nephrotic syndrome but they did not find any significant difference in the outcome of IgM-positive and IgM-negative patients [22]; however, it was found that the circulating IgG and IgM antibodies of PMN patients showed an altered pattern of reactivity to the self-antigens compared to the healthy controls in spite of their normal reactivity to the non-self-antigens [23]. Le Viet *et al.* studied the predictive value of serum IgA, IgG and IgM in PMN children. They reported median serum levels of 1.15, 2.23, and 1.7 g/L for IgA, IgG, and IgM respectively. Serum IgA and IgG levels in patients were significantly lower than healthy controls. Moreover, IgG amount had a positive predictive value for steroid-resistant nephrotic syndrome (SRNS). With the cutoff point of 2.04 g/L, this test had the sensitivity and specificity of 89.5% and 95.5%, respectively. The IgG/IgM ratio also showed a positive predictive value for SRNS (AUC=0.892, P <0.001). Therefore, serum IgG level and IgG/IgM ratio might be considered as predictive markers for steroid resistance in children with idiopathic nephritic syndrome [24]. Moreover, Branten *et al.* suggested urine IgG with sensitivity and specificity of 88% as a helpful marker in predicting renal failure in PMN patients; this could be used to make appropriate decisions on the dose and combination of immunosuppressive regime [25]. Our study also demonstrated higher serum levels of IgG4 in both steroid-responsive and refractory patients which decreased significantly following RTX administration. This finding is in concordance with most previous studies indicating the implication of IGg4 in pathogenesis of PMN and suggesting its diagnostic value. Despite elevated amount of IgG4, the IgG1 and IgG2 were found in lower amounts in steroid-resistant cases and were not affected by RTX. The decreased level of IgG1 and IgG2 in pre-RTX group might be attributed to the previous infusions of rituximab which had reduced B cells general population. IgA and IgM levels showed no significant difference between studied groups implying their irrelevance to disease.

Conclusion

In summary, evaluating the IgG4 level not only in tissue biopsies and urine but also in peripheral blood could be useful in predicting the responsiveness of PMN patients to the standard treatment. This could also be used during the follow up period of patients as elevated IgG4 levels might be suggestive of uncontrolled disease. Serum amounts of IgA, IgM, IgG1 and IgG2 do not appear to be associated with the pathogenesis of PMN whereas rituximab showed the least (or null) effect on serum IgM concentration.

Conflict of interest statement: None declared

References

1. Couser WG. Primary membranous nephropathy. *Clin J Am Soc Nephro*, 2017; 12(6): 983-997.
2. Cattran DC, and Brenchley PE. Membranous nephropathy: integrating basic science into improved clinical management. *Kidney Int,* 2017; 91(3): 566-574.
3. Chapter 7: Idiopathic membranous nephropathy. *Kidney Int Suppl*, 2012; 2(2): 186-197.
4. Debiec H, and Ronco P. Immunopathogenesis of membranous nephropathy: an update. *Semin Immunopathol* 2014; Springer.
5. Lai WL, *et al*. Membranous nephropathy: a review on the pathogenesis, diagnosis, and treatment. *J Formos Med Assoc* 2015; 114(2): 102-111.
6. Debiec H, and Ronco P. Nephrotic syndrome: A new specific test for idiopathic membranous nephropathy. *Nat Rev Nephrol* 2011; 7(9): 496.
7. Takahashi S., *et al*. Nephrotic syndrome caused by immune-mediated acquired LCAT deficiency. *Clin J Am Soc Nephro* 2013; 24(8): 1305-1312.
8. Tran TH, *et al*. Overview of current and alternative therapies for idiopathic membranous nephropathy. *Pharmacotherapy* 2015; 35(4): 396-411.
9. Remuzzi G, et al. Rituximab for idiopathic membranous nephropathy. *Lancet* 2002; 360(9337): 923-924.
10. Weiner GJ. Rituximab: mechanism of action. *Semin Hematol* 2010; 47(2): 115-23.
11. Fornoni A, *et al*. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med* 2011; 3(85): 85ra46-85ra46.
12. Tasaki M, *et al*. Rituximab treatment prevents the early development of proteinuria following pig-to-baboon xenokidney transplantation. *Clin J Am Soc Nephro* 2014; ASN.2013040363.
13. Fervenza FC, Sethi S, and Specks U. Idiopathic membranous nephropathy: diagnosis and treatment. *Clin J Am Soc Nephro* 2008; 3(3): 905-919.
14. Bazzi C, *et al*. Fractional excretion of IgG in idiopathic membranous nephropathy with nephrotic syndrome: a predictive marker of risk and drug responsiveness. *BMC Nephrol* 2014; 15(1): 74.
15. Lonnbro-Widgren J, *et al*. Glomerular IgG subclasses in idiopathic and malignancy-associated membranous nephropathy. *Clin Kidney J* 2015; 8(4): 433-439.
16. von Haxthausen F, *et al*. Antigen-Specific IgG Subclasses in Primary and Malignancy-Associated Membranous Nephropathy. *Front Immunol* 2018; 9: 3035.
17. Na W, *et al*. Dissecting the relationships of IgG subclasses and complements in membranous lupus nephritis and

idiopathic membranous nephropathy. *PloS One* 2017; 12(3): e0174501.
18. Huang CC, *et al.* IgG subclass staining in renal biopsies with membranous glomerulonephritis indicates subclass switch during disease progression. *Modern Pathol* 2013; 26(6): 799.
19. Kuroki A, *et al*. Glomerular and serum IgG subclasses in diffuse proliferative lupus nephritis, membranous lupus nephritis, and idiopathic membranous nephropathy. *Internal Med* 2002; 41(11): 936-942.
20. Liu L, *et al*. Expression of phospholipase A2 receptor and IgG4 in patients with membranous nephropathy. *Vasc Health Risk Manag* 2018; 14: 103.
21. Yeo M-k, *et al*. The usefulness of phospholipase A2 receptor and IgG4 detection in differentiation primary membranous nephropathy from secondary membranous nephropathy in renal biopsy. *Appl Immunohisto M M* 2018; 26(8): 591-598.
22. Juozapaite S, *et al*. The impact of IgM deposits on the outcome of Nephrotic syndrome in children. *BMC nephrol* 2017; 18(1): 260.
23. Stahl D, *et al*. Idiopathic membranous glomerulonephritis is associated with altered patterns of self-reactive IgM and IgG antibody repertoires. *Scand. J Immunol* 2001; 54(5): 534-542.
24. Le Viet T, *et al*. Serum IgG Level and IgG/IgM Ratio on Admission Predict Steroid-Resistant Response in Vietnamese Children with Idiopathic Nephrotic Syndrome. *Nephrourol Mon* (In Press).
25. Branten AJ, *et al*. Urinary excretion of β2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: a validation study. *Clin J Am Soc Nephro* 2005; 16(1): 169-174.