Article

Membranous glomerulonephritis: Role of Retinol-binding Protein in monitoring and prognostication.

Sadiq Mu'azu Maifata [1,2,3], Rafidah Hod [2], Fadhlina Zakaria [4] and Fauzah Abd Ghani [1,*]

1.   Histopathology Unit, Department of Pathology, Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia; teemoh87@gmail.com (S.M.M.); fauzah@upm.edu.my (F.A.G.)

2.   Physiology Unit, Department of Anatomy, Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor; 43400, Malaysia; rafidahhod@upm.edu.my

3.   Department of Physiology, Faculty of Basic Medical Science, College of Medicine, Federal University Lafia, Lafia, Nasarawa 950102, Nigeria

4.   Nephrology Unit, Department of Medicine, Faculty of Medicine and Health Science, Universiti Putra Malaysia, Serdang, Selangor 43400, Malaysia; n_fadhlina@upm.edu.my

 * Correspondence: fauzah@upm.edu.my; Tel.: +6013-6277576.

Abstract: Retinol binding protein (RBP) which was initially thought to be a biomarker for proximal convoluted tubule dysfunction, could be important in chronic kidney disease. Membranous glomerulonephritis (MGN) was considered one of the most common causes of chronic kidney diseases (CKD) and subsequent progression to End-stage Renal Disease (ESRD). As such, monitoring MGN subjects especially those at risk of progression to ESRD is quite important for prompt intervention and treatment decision. This study aimed at using a non-invasive biomarker (urine RBP) for effective monitoring and prognostication of MGN subjects using the Enzyme-linked Immunosorbent Assay (ELISA) method. One hundred and twenty-five, including 81 primary and 44 secondary MGN subjects, were diagnosed with MGN from January 2012 to October 2019 at Hospital Serdang and Hospital Kuala Lumpur from which 69 subjects consisting of 45 primary and 24 secondary MGN subjects participated in the study. The test for biomarkers using the ELISA technique gave the following results: urine RBP was detected in 27 (39.1%) and 6 (8.7%) primary and secondary MGN subjects respectively. Correlation analysis shows a significant correlation between urinary RBP and renal function test parameters, in addition to logistic regression analysis which demonstrated that urinary RBP is a prognostic biomarker for primary MGN. Therefore, urinary RBP could be used in monitoring and prognostication of primary MGN subjects.

Keywords: Membranous glomerulonephritis (MGN); Retinol binding protein (RBP); Prognostication; End-stage renal diseases

1. Introduction

Retinol binding protein is a low molecular weight protein, synthesized in the liver (1), binds to transthyretin (a complex that prevents glomerular filtration), and is found in plasma circulation (2). The principal function of RBP is vitamin A transport (3). It has been documented that about 4-5% of RBP circulates freely, crosses the glomerular barrier and is

reabsorbed at the proximal convoluted tubule (PCT) (4).   Individualized therapy is possible with the development of a non-invasive biomarker such as urinary RBP to monitor chronic kidney disease (CKD) patients at risk of progressing to end-stage renal disease (ESRD) (5,6). Besides, urinary RBP is linked to a high risk of creatinine doubling and renal replacement therapy initiation in CKD (7). Therefore, urinary RBP could serve as a non-invasive biomarker for PCT dysfunction (8) and CKDs (4,9,10) such as diabetes mellitus (DM), HIV-associated nephropathy (HIVAN) for effective intervention (11). Membranous glomerulonephritis (MGN) is one of the leading causes of CKD and progression to ESRD is linked to high morbidity and mortality (4,12). Therefore, early intervention and monitoring are necessary to prevent patients from progressing to ESRD. Previous researches demonstrated that higher urinary RBP is related to the possibility of ESRD and corticosteroid tolerance in acute glomerulonephritis (13,14).

2. Materials and Method

2.1. Study design and population

This is a retrospective study design involving MGN patients on follow-up at Hospital Serdang and Hospital Kuala Lumpur. The National Medical Research Register Malaysia approved the study (NMRR-18-3245-44092).

2.2. Method

2.2.1. Collection of data

Immunohistochemical (IHC) stain for PLA$_2$R and THSD7A and clinical parameters conducted on the same patients were used to define MGN as either primary or secondary. Of the 125 MGN patients, 69 of them were consented and participated in the study.

The urine RBP was detected using the RBP4 ELISA kit obtained from Abcam-ab19624 and the standard was obtained as in appendix 1. Besides, urine and serum samples from the patients were sent for serum albumin, serum creatinine, serum urea and UPCr index while eGFR was calculated by CKD-EPI Creatinine 2009 equation.

2.2.2. The references for the laboratory parameters

  Low serum albumin was defined as < 35g/L, serum creatinine as 44-80mol/L and serum urea as 2.76-8.07mmol/L. The primary outcomes were defined as eGFR < 60 ml/min/1.73m^2 (CKD≥ 3) and UPCr Index > 0.03 g/mmol (no remission) while the reference value for urinary RBP was 0.86 pg/mL (optimized by ROC curve).

2.3. Analysis of data

The statistical analysis was performed through version 25.0 of the standard software package (SPSS). The mean, the standard deviation was used for normally distributed variables, while the median (interquartile) was used for variables that are not normally distributed. Categorical variables were represented as frequencies and percentages and compared by chi-square. Simple and multiple regressions were conducted to determine the primary outcome, and the difference was found to be statistically significant at $p < 0.05$.

3. The results

3.1. Characteristics of the patients at presentation (n= 69)

Sixty-nine patients, including 47 primary MGN and 22 secondary MGN, were involved in this research. Table 1 shows that primary and secondary MGN were more common among patients 30-40 years of age, with 26 of 47 male patients associated with primary MGN, while 14 of 22 female patients were associated with secondary MGN. In addition, Malay and Chinese were more associated with primary MGN, while secondary MGN was mostly seen among Malay patients. Nephrotic range proteinuria (38 of 47) and hypertension (28 of 47) have been seen more frequently in primary MGN patients.

Table 1. Characteristics of patients (n = 69) at the time of renal biopsy

variables	Primary MGN		Secondary MGN	
	Frequency (n=47)	Percentage (%)	Frequency (n= 22)	Percentage (%)
Age at biopsy:				
30-40	24	51.1	11	50.0
41-60	16	34.0	5	22.7
>60	7	14.9	6	27.3
Gender:				
Male	26	55.3	8	59.1
Female	21	44.7	14	40.9
Race:				
Malay	24	51.1	16	72.7
Chinese	12	25.5	5	22.7
others	11	23.4	1	4.6
Nephrotic range proteinuria:				
Yes	38	80.8	13	59.1
No	9	19.1	9	40.9
Hypertension:				
Yes	28	59.6	10	45.5
No	19	40.4	12	54.5
Haematuria:				
Yes	9	19.1	2	9.1
No	38	80.9	20	90.9

3.2. The patients' outcome after the follow-up period.

At the end of the follow-up period, 14 (66.7%) of the primary MGN have lower eGFR (high risk) compared to 7 (33.3%) of the secondary MGN. Also, 61.4% of primary MGN patients could not achieve remission as compared to 38.6% of secondary MGN, although not statistically significant. Also, Table 2 denoted that the urinary RBP was detected in 27 and 6 primary and secondary MGN patients respectively and that primary MGN was more associated with urinary RBP (pg/mL) compared to those with secondary MGN (X^2= 5.468, p= 0.019).

Table 2: The patients' outcome after the follow-up period.

variables	Primary MGN n=47 (%)	Secondary MGN n= 22 (%)	X^2	p-value
		Median (Interquartile)		
Follow-up period (month)	39.0(17.5-59.5)	27.5(13.0-49.8)		
eGFR (ml/min/1.73m^2)			0.0292	0.863
High risk	14(66.7)	7(33.3)		
Low risk	33(68.7)	15(31.3)		
UPCr Index(g/mmol)			2.549	0.110
Remission	20(80.0)	5(20.0)		
No remission	27(61.4)	17(38.6)		
Urinary RBP (pg/ml)			5.468	**0.019***
Positive	27(81.8)	6(18.2)		
Negative	20(55.6)	16(44.4)		

High risk eGFR (< 60 ml/min/1.73m^2), Low risk eGFR (> 60 ml/min/1.73m^2), categorical variables were expressed as frequency and percentages and compared by chi-square(X^2), statistical significance level $p< 0.05$*

3.3. Relationship between urinary RBP and renal function test parameters at the end of the follow-up period

Correlation analysis between RBP level in urine and renal function test parameters in Table 3 shows a good positive significant correlation with serum urea in mmol/L (r= 0.500, p< 0.05), serum creatinine in μmol/L (r= 0.534, p <0.05) while a negative significant relationship was obtained in the case of eGFR ml/min/1.73m^2 (r= -0.734, p <0.05) and a poor positive significant relationship with UPCr Index in g/mmol (r =0.235, p= 0.049) was recorded.

Urinary RBP was also correlated with anti-PLA$_2$R and anti-THSD7A antibodies (Abs) from different studies but conducted on the same patients. The results show a poor significant correlation with anti-PLA$_2$R Abs (r= 0.239, p 0.042) with no significant relationship with anti-THSD7A Abs. The graphical representation of the correlations was presented in appendix 2.

Table 3. Correlation analysis between urinary RBP and renal function test parameters

Variables	r	p
Urinary RBP (pg/ml)		
Serum albumin (g/L)	0.046	0.705
Serum urea(mmol/L)	0.500	**<0.05**
Serum creatinine (μmol/L)	0.534	**<0.05**
eGFR (ml/min/1.73m^2)	-0.734	**<0.05**
UPCr Index (g/mmol)	0.235	**0.049***
Serum anti-PLA$_2$R Abs (ng/ml)	0.239	**0.042***
Serum anti-THSD7A Abs (ng/ml)	0.054	0.825

Correlation (r) given as; < 0.25 (poor), 0.26-0.51(fair), 0.51-0.75 (good) and > 0.75 (excellent), level of significance < 0.05*.

3.5. The outcome of MGN patients at the end of follow-up

3.5.1. The outcome of primary MGN patients using eGFR (< 60 ml/min/1.73m^2)

A prognostic outcome for chronic kidney disease was determined using eGFR (< 60 ml/min/1.73m^2); most variables except gender and albumin were preserved following a simple logistic regression as defined in Table 4. However, a

variable such as serum creatinine was exempted owing to multicollinearity. During multiple logistic regression, only serum urea (95% C. I= 0.130-0.734, AOR= 0.309, p= 0.008) and urinary RBP (95% C. I= 0.00-0.549, AOR= 0.026, p= 0.026) as shown in Table 5. For any rise in serum urea in mmol/l, the risk of low eGFR (< 60 ml/min/1.73m^2) increases by 3.236. In contrast, high urinary RBP titre in pg/ml increases the likelihood of a primary outcome (< 60 ml/min/1.73m^2) by an odd of 12.987 compared to those with normal urinary RBP titre.

Table 4. The simple logistic regression for the outcome of primary MGN using eGFR (< 60 ml/min/1.73m^2)

Variables	B-coefficient	S. E	COR	C. I (95%)	P
Age at biopsy (years)	0.062	0.023	0.940	0.898-0.993	**0.008***
Gender:					
Male			1.000		
Female	0.241	0.587	1.273	0.403-4.021	0.681
Serum albumin (g/l)	0.111	0.587	1.273	0.961-1.299	0.149
Serum urea (mmol/l)	0.883	0.259	2.417	1.454-4.029	**0.002***
Serum creatinine (μmol/L)	0.178	0.068	1.195	1.046-1.316	**0.009***
UPCr Index(g/mmol)					
Remission achieved			1.000		
No remission	-2.565	0.813	0.077	0.016-0.378	**0.002***
Urinary RBP (pg/ml)					
Negative			1.000		
Positive	-3.250	0.813	0.077	0.160-0.378	**0.002***

S. E= standard error, COR= crude odd ratio, C. I= confidence interval, level of significance p< 0.05*

Table 5. The multiple logistic regression for the outcome of primary MGN using eGFR (< 60 ml/min/1.73m^2)

Variables	B-coefficient	S. E	AOR	95% C. I	P
Age at biopsy (years)	-0.094	0.052	0.910	0.822-1.009	0.534
Serum urea (mmol/l)	-1.174	0.441	0.309	0.130-0.734	**0.008***
Urinary RBP (pg/ml)					
Negative			1.000		**0.026***
Positive	-4.987	2.239	0.007	0.00-0.549	

S. E= standard error, AOR= adjusted odd ratio, C. I= confidence interval, level of significance p< 0.05*

3.5.2. The outcome of primary MGN using UPCr Index (No remission) at the end of the follow-up period.

A renal function test parameter for remission (UPCr Index) was used as a primary outcome to determine the prognosis of primary MGN, as shown in Tables 6 and 7. The simple logistic regression conducted demonstrated that serum creatinine (95% C. I= 1.016-1.079, COR= 1.047, p= 0.002), eGFR (95% C. I= 0.931-0.978, COR= 0.954, p < 0.05) and urine RBP (95% C. I= 3.513-50.315, COR= 13.295, p < 0.05) were retained. Following multiple regression analysis, only urinary RBP (95% C. I= 2.570-48.672, AOR= 11.183) was significantly associated with not attending remission at the end of the follow-up period. This implies that for any unit rise in urinary RBP, the risk of not achieving remission increases by an odd of 11.183.

Table 6. The simple logistic regression for the outcome of primary MGN using UPCr Index (No remission)

Variables	B-coefficient	S. E	COR	95% C. I	P
Age at biopsy (years)	0.025	0.018	1.025	0.989-1.063	0.181
Gender:					0.530
Male			1.000		
Female	0.308	0.490	1.360	0.521-3.551	
Serum albumin (g/l)	-0.075	0.060	0.928	0.825-1.043	0.209
Serum urea (mmol/l)	0.257	0.136	1.293	0.991-1.686	0.058
Serum creatinine (μmol/L)	0.046	0.015	1.047	1.016-1.079	**0.002***
eGFR (ml/min/1.73m²)	-0.047	0.013	0.954	0.931-0.978	**<0.05***
Urinary RBP (pg/ml)	2.587	0.679	13.295	3.513-50.315	**<0.05***

S. E= standard error, COR= crude odd ratio, C. I= confidence interval, level of significance p< **0.05***

Table 7. The multiple regression analysis for the outcome of primary MGN using UPCr Index (No remission).

variables	B-coefficient	S. E	AOR	95% C. I	P-value
Serum creatinine	0.018	0.044	1.018	0.934-1.110	0.660
eGFR(ml/min/1.73m²)	0.002	0.039	1.002	0.928-1.882	0.958
Urinary RBP (pg/ml)	2.414	0.750	11.183	2.570-48.672	**0.049***

S. E= standard error, AOR= adjusted odd ratio, C. I= confidence interval, level of significance p< **0.05***

4. Discussion

The importance of urinary RBP in determining the prognosis of primary MGN compared to secondary MGN was emphasized in this study.

Sixty-nine patients comprising 47 primary and 22 secondary MGN were enrolled in this research. The research emphasized the function of urinary RBP as a prognostic biomarker for chronic kidney disease. It may play a role in the monitoring of those primary MGN patients at risk of ESRD progression. This is in complement with a similar study conducted on the same patients, which denoted the importance of anti-PLA2R and anti-THSD7A in determining the prognosis of primary MGN patients (15).

4.1. Role of urinary RBP in monitoring primary MGN patients

A laboratory parameter such as eGFR, known to be used in monitoring MGN patients at risk of progressing to ESRD using CKD classification (16,17) was found to have a significant negative relationship with urine RBP. This implies that lower eGFR is associated with a higher urine RBP titre. Also, UPCr Index used in monitoring primary MGN subjects (especially those presenting with nephrotic syndrome) is significantly correlated with urine RBP. It was also reported that high urinary levels of RBP predicted poor proteinuria outcomes (18). This demonstrated that urinary RBP could be used in monitoring MGN subjects and in the treatment decision in patients with primary MGN.

4.2. Urine RBP and prognosis

RBP was discovered to be a reliable biomarker for MGN, predicting those at risk of ESRD (1). In this study, a high titre of urinary RBP was detected more frequently among primary MGN subjects with lower eGFR, higher creatinine and urea levels. Regression analysis conducted to detect factors associated with progression to ESRD in primary MGN subjects demonstrated the persistence of urinary RBP as the main predictor of progression to ESRD. This finding was also supported by a similar study which predicted that urinary RBP could serve as a biomarker for general kidney diseases and not only the proximal convoluted tubule dysfunction (1). Regression analysis in tables 6 and 7 suggested that urinary RBP is associated with achieving remission. Therefore, urinary RBP could serve as an important biomarker for the prognosis of primary MGN. Therefore, higher urinary RBP titre is associated with remission and vice versa.

5.   Conclusions

Urine RBP could be useful in monitoring and prognostication of primary MGN patients at risk of progression to ESRD.

6.   Contributions of authors:

The conceptualization was done by Sadiq Mu'azu Maifata and Fauzah Abd Ghani.; methodology, Sadiq Mu'azu Maifata and Fauzah Abd Ghani; validation, Nor Fadhlina Zakaria., Fauzah Abd Ghani and Rafidah Hod; formal analysis, Sadiq Mu'azu Maifata; investigation, Nor Fadhlina and Sadiq Mu'azu Maifata; resources, Fauzah Abd Ghani; data curation, Sadiq Mu'azu Maifata.; writing—original draft preparation, Sadiq Mu'azu Maifata; writing—review and editing, Nor Fadhlina Zakaria, Rafidah Hod and Fauzah Abd Ghani; visualization, Sadiq Mu'azu Maifata; supervision, Fauzah Abd Ghani, Rafidah Hod and Nor Fadhlina Zakaria; project administration, Fauzah Abd Ghani; funding acquisition, Fauzah Abd Ghani and Nor Fadhlina Zakaria.

8.   References

1.   Domingos MAM, Moreira SR, Gomez L, Goulart A, Lotufo PA, Benseñor I, Titan S. Urinary retinol-binding protein: Relationship to renal function and cardiovascular risk factors in chronic kidney disease. PLoS One. 2016;11(9):1–10.

2.   Peterson A P. Studies on the interaction between pre-albumin, retinol-binding protein and vitamin A. Biol Chem. 1971;246(1):44–9.

3.   Kawaguchi R, Zhong M, Kassai M, Ter-Stepanian M, Sun H. Vitamin a transport mechanism of the multitransmembrane cell-surface receptor STRA6. Membranes (Basel). 2015;5(3):425–53.

4.   Maifata SM, Hod R, Zakaria NF, Ghani FA. Membranous Glomerulonephritis: Overview of the Role of Serum and Urine Biomarkers in patients' Management. Biomedicine. 2019;7:86.

5.   Weiss RH & Kim K. Metabolomics in the study of kidney diseases. Nat Rev Nephrol. 2011;8:22–3.

6.   Cravedi P, Ruggenenti P RG. Proteinuria should be used as a surrogate in CKD. Nat Rev Nephrol. 2013;8:301–6.

7.   Titan SM, Vieira JM Jr, Dominguez WV, Moreira SR, Pereira AB, Barros RT, Zatz R.. Urinary MCP-1 and RBP: Independent predictors of renal outcome in macroalbuminuric diabetic nephropathy. J Diabetes Complications [Internet]. 2012;26(6):546–53. Available from: http://dx.doi.org/10.1016/j.jdiacomp.2012.06.006

8.   Lopez-Giacoman S. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol. 2015;4(1):57.

9.   Li A, Yi B, Liu Y, Wang J, Dai Q, Huang Y, Chun LY, Zhang H . Urinary NGAL and RBP Are Biomarkers of Normoalbuminuric Renal Insufficiency in Type 2 Diabetes Mellitus. J Immunol Res. 2019;2019.

10. Qin Y, Zhang S, Shen X, Zhang S, Wang J, Zuo M, Xiao C, Gao Z, Yang J, Zhu H, Baocheng C. Evaluation of urinary biomarkers for prediction of diabetic kidney disease: a propensity score matching analysis. Ther Adv Endocrinol Metab. 2019;10:1–11.

11. Bazzi C, Bakoush O. Proteinuric Biomarkers in Chronic Kidney Disease. Biomarkers Kidney Dis. 2016;(June):1–20.

12. Braun N, Schweisfurth A, Lohöfener C, Lange C, Gründemann C, Kundt G, Gröne HJ. Epidemiology of glomerulonephritis in Northern Germany. Int Urol Nephrol. 2011;43(4):1117–26.

13. Kirztajn GM, Nishida SK, Silva MS, Ajzen H, Moura LA, PA. Urinary retinol-binding protein as a prognostic

marker in glomerulopathies. Nephron. 2002;90(4):424–31.

14.   Ricardo Sesso, Alize P. Santos, Sonia K. Nishida MJK, Joao T. Carvalhaes, Horacio Ajzen OLR and ABP. Prediction of Steroid Responsiveness in the Idiopathic Nephrotic Syndrome Using Urinary Retinol-binding Protein and Beta-2-Microglobulin. Ann Intern, Med. 1992;116:905-909.

15.   Maifata SM, Hod R, Zakaria F, Ghani FA. Role of Serum and Urine Biomarkers (PLA2R and THSD7A) in Diagnosis, Monitoring and Prognostication of Primary Membranous Glomerulonephritis. Biomolecules. 2020;10(2):319.

16.   Alp Ikizler T. CKD classification: Time to move beyond KDOQI. J Am Soc Nephrol. 2009;20(5):929–30.

17.   Weiner DE, Krassilnikova M, Tighiouart H, Salem DN, Levey AS, Sarnak MJ. CKD classification based on estimated GFR over three years and subsequent cardiac and mortality outcomes: A cohort study. BMC Nephrol. 2009;10(1):1–11.

18.   Lin L, Ming Wang W, Xia Pan X, Xu J, Ni Gao C, Zhang W, Ren H, Xie JY, Shen PY, Xu YW, Ni LY and Nan Chen. Biomarkers to detect membranous nephropathy in Chinese patients [Internet]. Vol. 7, Nephrology Dialysis Transplantation. Elsevier Inc; 2016. Available from: http://www.jasn.org/cgi/doi/10.1681/ASN.2014111061

19.   Maria Alice Muniz Domingos, Silvia Regina Moreira, Luz Gomez, Alessandra Goulart, Paulo Andrade Lotufo, Isabela Benseñor ST. Urinary Retinol-Binding Protein: Relationship to Renal Function and Cardiovascular Risk Factors in Chronic Kidney Disease. PLoS One. 2016;11(9):0162782.