Urinary orosomucoid and retinol binding protein levels as early diagnostic markers for diabetic nephropathy

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Abstract

Background:

Diagnosing diabetic nephropathy is important to prevent long-term kidney damage and determine the prognosis of patients with diabetes. Since some kidney injury biomarkers increase in the early stages of diabetic nephropathy, this study investigated the clinical significance of combined detection of urine orosomucoid and retinol binding protein for early diagnosis of diabetic nephropathy.

Methods:

We recruited 72 patients with type 2 diabetes and 34 healthy persons from August 2016 to July 2018 at our hospital. Using Mogensen grading criteria, participants were classified as diabetes or diabetic nephropathy, and healthy persons constituted the control group. Urine orosomucoid and retinol binding protein were measured and correlated with other variables.

Results:

Increase in renal damage raised urinary orosomucoid level gradually ($P < 0.05$). Urinary retinol binding protein and microalbumin levels were significantly higher in the diabetes group than in control and nephropathy groups. Orosomucoid and retinol binding protein might be independent risk factors for diabetes and diabetic nephropathy. Urinary orosomucoid significantly correlated with retinol binding protein and microalbumin ($r = 0.489$ and 0.513, respectively) in the diabetic nephropathy group. The receiver operating characteristic curve yielded a sensitivity, specificity, and correction index of 0.941, 0.842, and 0.783, respectively, while analysis for retinol binding protein yielded a sensitivity of 0.942, specificity of 1.000, and a correction index of 0.941.

Conclusion:

The increase in the levels of urine orosomucoid and retinol binding protein can be detected in the early stages of type 2 diabetic nephropathy. Both these markers are important for diabetic nephropathy detection and early treatment.

Introduction

Diabetic nephropathy (DN) is the most common and severe chronic vascular complication among patients with type 2 diabetes mellitus (T2DM) [1]. It leads to chronic renal failure and is the leading cause of death due to diabetes. However, DN often occurs with no obvious symptoms in the early stage. Although renal biopsy is the most important diagnostic method for DN, it is associated with some trauma; as most patients find it difficult to endure, it cannot be readily employed for routine examination. Common diagnostic indicators for DN include 24-hour urine microalbumin (MAL), urea nitrogen, and serum creatinine. However, they can be affected by many factors, such as urinary tract or systemic infections, strenuous exercise, bleeding, or drugs that affect the kidneys [2]. The accuracy and specificity

of these indicators are not high, and they have limitations [3]; thus, more research is needed to identify newer, more accurate, and specific early diagnostic markers of DN. At present, preliminary progress has been made in kidney disease research using proteomics technology. Diabetic urine proteome research has shown that some protein markers have a predictive effect for early DN [4, 5]. In this study, the urine orosomucoid and retinol binding protein (RBP) levels were measured in healthy people, patients with T2DM, and patients with early DN. The differences between the three groups were compared. We also assessed their clinical significance in the diagnosis of early type 2 DN and their clinical value in the progression of nephropathy.

Materials And Methods

Study subjects

Thirty-four healthy people who underwent physical examination at our hospital from August 2016 to July 2018 were categorized as the normal control (NC) group, which included 18 males and 16 females, with an average age of 47.9 ± 14.2 years. Seventy-seven patients with T2DM who were hospitalized at the same time were assessed according to the Mogensen classification criteria for degree of kidney damage. These patients were categorized as those with T2DM (T2DM group; n = 38; microalbumin (MAL) < 30 mg/24 h), which included 21 males and 17 females, with an average age of 48.7 ± 13.6 years, patients with early DN and combined diabetic retinopathy, (T2DN group; n = 34; MAL 30–300 mg / 24 h), which included 19 males and 15 females, with an average age of 49.1 ± 14.4 years. Diabetes was diagnosed and classified according to the 1999 diagnostic criteria of the World Health Organization [6]. The exclusion criteria were patients with diabetic ketosis, hyperglycemia and osmotic pressure syndrome, combined fever and infection, acute cardiocerebrovascular diseases and urinary system diseases (kidney stones, acute and chronic nephritis, and nephrotic syndrome), non-diabetic congestive heart failure, liver dysfunction, rheumatic diseases, hematological diseases, pregnancy, tumors, fractures, primary hyperparathyroidism, a history of kidney transplant and intake of glucocorticoids, history of immunosuppressant and nephrotoxic drugs use, history of renal damage caused by strenuous exercise and severe hypertension.

Data Collection

We recorded the medical history of all patients, and measured their diastolic blood pressure (DBP), systolic blood pressure (SBP), height (cm), and weight (kg). The levels of total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), fasting blood glucose (FBG), 2-hour blood glucose, glycosylated hemoglobin (HbA1c) and serum creatinine (SCr) were measured. Urine was collected from 10 pm to 6 am the following morning, and the total urine volume (mL) was recorded after mixing. The urine MAL level was assessed for 24 hours. The urine orosomucoid and RBP levels were measured by the ELISA method. The MDRD equation [7] was used to estimate the glomerular filtration rate (eGFR).

Statistical analysis

For normally distributed data as determined by the Shapiro-Wilk's test, the indicators in each group were expressed as the mean ± standard deviation. Chi-squared tests were used to compare quantitative data between groups. The mean values for each of the three groups were compared using one-way analysis of variance. If there were significant differences between the groups, intra-group comparisons were performed using least significant difference. A binary logistic regression model was used to determine the factors associated with T2DM and T2DN, and correlation analyses were performed using Spearman's rank correlation. The receiver operating characteristic (ROC) curve was used to analyze the diagnostic points and diagnostic value of orosomucoid and RBP in DN. All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). A two-tailed test with $P < 0.05$ was considered statistically significant.

Results

There were no significant differences in age and gender among the three groups ($P > 0.05$).There were no statistically significant differences in DBP, BMI, CHOL, TG, and LDL levels among the three groups ($P > 0.05$) (Table 1).

Table 1
Comparison of general clinical data among the three groups

Groups	NC (n = 34)	T2DM (n = 38)	T2DN (n = 34)	F	P
BMI, kg/m^2	26.19 ± 1.28	26.32 ± 1.31	26.27 ± 1.24	0.094	0.911
SBP, mmHg	117.82 ± 12.06	130.61 ± 12.44[a]	142.55 ± 14.78[ab]	33.11	< 0.001
DBP, mmHg	80.48 ± 9.16	83.26 ± 9.36	85.19 ± 10.08	2.098	0.128
HbA1c (%)	5.3 ± 0.51	10.45 ± 2.65[a]	10.27 ± 2.77[a]	58.55	< 0.001
FBG, mmol/L	4.63 ± 0.52	9.98 ± 4.63[a]	10.69 ± 4.27[a]	27.710	< 0.001
CHOL, mmol/L	4.35 ± 0.66	4.82 ± 1.08	4.83 ± 1.14	2.670	0.074
TG, mmol/L	1.29 ± 0.54	1.3 ± 0.61	1.33 ± 0.56	0.045	0.956
LDL, mmol/L	2.17 ± 0.43	2.33 ± 0.61	2.41 ± 0.69	1.474	0.234
[a]Compared with NC: $P < 0.05$,					
[b]Compared with T2DM: $P < 0.05$					
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c (%), glycosylated hemoglobin; FBG, mmol/L, fasting blood glucose; CHOL, mmol/L, cholesterol; TG, mmol/L, triglycerides; LDL, mmol/L, low density lipoproteins.					

There were significant differences in SBP, HbA1c, and FBG between the NC and the other two groups ($P < 0.05$). However, there was no statistical difference in the general clinical data between the T2DM and T2DN groups ($P > 0.05$). As renal damage increased in patients, urine orosomucoid levels gradually increased as well ($P < 0.05$) (Table 2).

Table 2
Comparison of urinary orosomucoid, RBP, MAL, and eGFR levels among the three groups

Groups	NC (n = 34)	T2DM (n = 38)	T2DN (n = 34)	F	P
Orosomucoid, mg/L	9.45 ± 2.03	18.35 ± 4.04[a]	29.46 ± 6.13 [ab]	177.82	< 0.001
RBP, mg/L	0.26 ± 0.07	0.31 ± 0.09	0.95 ± 0.28 [ab]	172.56	< 0.001
eGFR, mL/min/1.73 m^2	108.08 ± 13.73	102.17 ± 10.12	94.92 ± 10.57[ab]	11.11	< 0.001
MAL, mg/24 h	10.22 ± 6.42	12.13 ± 7.83	199.65 ± 49.72[ab]	492.19	< 0.001
[a]Compared with NC group: $P < 0.05$					
[b]Compared with T2DM group: $P < 0.05$					
Abbreviations, RBF, renal blood flow; eGFR, estimated glomerular filtration rate; MAL, microalbumin.					

Urine RBP and MAL levels in the T2DN group were significantly higher than those in the NC and T2DN groups ($P < 0.001$). The eGFR levels in the T2DN group were significantly lower than in the NC and T2DM groups ($P < 0.001$). There were no significant differences in RBP, eGFR, and MAL levels between the NC and T2DM groups ($P > 0.05$).

For the NC group and the T2DM group, the dependent variable was whether T2DM had occurred (Yes = 1, No = 0), and the independent variables were the four variables with differences between the two groups, shown in Tables 1 and 2, including SBP, HbA1c, FBG, and orosomucoid. A binary logistic regression model was established and used to determine the influence of these four variables on T2DM (Table 3), and all were shown to be risk factors (all $OR > 1$, $p < 0.05$).

Table 3
Binary logistic regression analysis of the factors associated with type 2 diabetes mellitus

Variable	Regression coefficient (B)	Significance level (P)	Odds ratio (OR)	95% CI of the OR	
				Lower limit	Upper limit
SBP, mmHg	0.106	0.000	1.112	1.056	1.170
HbA1c (%)	2.133	0.001	8.438	2.320	30.688
FBG, mmol/L	1.022	0.000	2.779	1.663	4.646
Orosomucoid, mg/L	0.964	0.001	2.621	1.521	4.516

Abbreviations: SBP, systolic blood pressure; HbA1c, glycosylated hemoglobin; FBG, fasting blood glucose.; OR, odds ration; CI, confidence interval.

For the T2DM group and the T2DN group, the dependent variable was whether T2DN had occurred (Yes = 1, No = 0), and the independent variables were the five variables with differences between the two groups shown in Tables 1 and 2. A binary logistic regression model was established for analysis (Table 4).

Table 4
Binary logistic regression analysis of the factors associated with Type 2 diabetic nephropathy

Variable	Regression coefficient (B)	Significance level (P)	Odds ratio (OR)	95% CI of the OR	
				Lower limit	Upper limit
SBP, mmHg	0.089	0.000	1.093	1.045	1.143
Orosomucoid, mg/L	0.626	0.000	1.871	1.360	2.574
RBP, mg/L	0.241	0.023	13.305	9.079	26.000
eGFR, mL/min/1.73 m^2	-0.054	0.021	0.948	0.905	0.992
MAL, mg/24 h	0.892	0.000	2.441	1.070	3.149

Abbreviations: RBF, renal blood flow; eGFR, estimated glomerular filtration rate; MAL, microalbumin; SBP, systolic blood pressure; OR, odds ration; CI, confidence interval.

Of the five factors that were included in the regression model ($p < 0.05$), SBP, orosomucoid, RBP, and MAL were all determined to be risk factors (OR > 1) and eGFR was shown to be a protective factor (OR = 0.948 > 1). Correlation analysis showed that in the T2DN group, urinary orosomucoid level was significantly positively correlated with RBP ($r = 0.489$) and MAL ($r = 0.513$). RBP and MAL were significantly positively

correlated with a correlation coefficient of 0.468. eGFR and urine orosomucoid, RBP, and MAL were significantly negatively correlated (r = -0.577, -0.474, and − 0.466, respectively).

ROC curve analysis was used to assess the diagnostic points and diagnostic value of orosomucoid and that of RBP to predict DN. Figure 1 and Table 5 show the areas under the ROC curves for orosomucoid and RBP with the respective standard error values.

Table 5
Areas under the two ROC curves for predicting diabetic nephropathy

Variable	Area under the ROC curve	Standard error	P	95% confidence interval	
				-LR	+LR
Orosomucoid	0.953	0.021	0.00	0.875	0.989
RBP	0.970	0.022	0.00	0.900	0.996

Abbreviations: RBP, renal blood flow; ROC, receiver operating characteristic; -LR, negative likelihood ratio; LR, positive likelihood ratio.

The diagnostic value of DN had improved; however, no statistical significance was observed (Z = 0.598, P = 0.550 > 0.05). The diagnostic point of orosomucoid was 22.43, sensitivity was 0.941, specificity was 0.842, and Youden index was 0.783. The diagnostic point of RBP was 0.53, sensitivity was 0.942, specificity was 1.000, and Youden index was 0.941.

Discussion

DN has become the leading indication for dialysis due to end-stage renal disease (ESRD) [8, 9]. Recent findings suggest that immune-mediated inflammatory processes play a crucial role in DN. Many pre-inflammatory cells, growth regulators, and adhesion factors interact with each other and cross-link, resulting in an expansion of the corresponding cascade of inflammation [10]. In recent years, the rapid development of proteomics technology has provided us with new methods and ideas for identifying early diagnostic markers of DN. Proteomics techniques have been used to identify disease-specific biomarkers and other related proteins in urine. Differential proteins have been identified, and some protein markers were found to have predictive effects for glomerular diseases.

Orosomucoid protein is a non-specific acute phase reaction protein that is mainly synthesized and secreted by the liver, is low in healthy body fluids, but is significantly increased in a state of inflammation or for people with tumors. Orosomucoid can act in damaged areas, be released into the circulation and intercellular fluid, and become involved in the induction and regulation of body damage, immune, and inflammatory responses [11]. Elevated urine orosomucoid levels in T2DM patients have predictive effects on cardiovascular complications and mortality [12]. El-Beblawy et al. [13] pointed out that orosomucoid is an independent factor of diabetic microvascular complications and can be considered as an early marker of kidney damage. Fandiño-Vaquero et al. [14] also found that orosomucoid levels increased in patients

with T2DM, and that it might mirror local endothelial dysfunction or inflammatory processes. Although there exists research and knowledge about orosomucoid, research on changes in orosomucoid concentration in urine during the early stage of DN is lacking. In this study, we found that as the disease progressed, urine orosomucoid levels gradually increased ($P < 0.001$). Orosomucoid might be an independent risk factor for T2DM and T2DN, and it had a significant positive correlation with MAL ($r = 0.489$) and a significant negative correlation with eGFR ($r = -0.577$). The results also revealed an increase in orosomucoid in the early stage of DN, suggesting that this increase may promote the occurrence and development of DN.

RBP is filtered through the glomerulus and absorbed and degraded by proximal tubular epithelial cells. Therefore, it is generally stable in urine, difficult to decompose, and has a low excretion rate. An increase in RBP excretion may reflect the extent of damage [15, 16]. Studies have shown that urine RBP levels in patients with T2DM are closely related to DN [17, 18]. Some studies have shown that urine RBP can be used to assess the degree of renal interstitial fibrosis due to various causes, progress with ESRD dialysis, and even diabetes related to increased risk of death [19, 20]. In this study, we found that urine RBP and MAL levels in the T2DN group were significantly increased ($P < 0.001$). Therefore, RBP might be an independent risk factor for T2DN, as it had a significant positive correlation with MAL ($r = 0.468$) and a significant negative correlation with eGFR ($r = -0.474$). Urine RBP may reflect early renal damage in DN. In the area under the ROC curve for predicting DN by orosomucoid and RBP both factors have high sensitivity and specificity. Therefore, both orosomucoid and RBP can be used to diagnose DN.

The main limitation of this study is that all the participants were residents of Henan Province, China, and the sample size was small. Further verification is needed through large sample sizes and multicenter studies.

Conclusions

In summary, urine orosomucoid and RBP may be new markers for the early diagnosis of DN, which is of great significance for the early detection and treatment of DN. However, the underlying molecular mechanisms and the clinically important levels of these potential biomarkers need to be studied further.

Abbreviations

AKI Acute Kidney Injury

BMI Body mass index

CHOL Cholesterol

CI Confidence interval

DBP Diastolic blood pressure

DN Diabetic nephropathy

ESRD End-stage renal disease

FBG Fasting blood glucose

LDL Low-density lipoprotein

MAL Microalbumin

NC Normal control

NGAL Neutrophil gelatinase-associated lipocalin

RBF Renal blood flow

RBP Retinol binding protein

ROC Receiver operating characteristic

SBP Systolic blood pressure

Declarations

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of The First Affiliated Hospital of Henan Polytechnic University (Jiaozuo Second People's Hospital) (IRB number: 2016010), and all patients provided written informed consent to participate in the study.

Consent for publication

Consent publication was provided.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request and with the permission of The First Affiliated Hospital of Henan Polytechnic University ethics committee.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SY L analyzed and interpreted the patient data regarding diabetic nephropathy and the normal control. LF conducted serological testing and analysis. BY, YF L, XQ W, JC, and HH W screened the hospitalized patients and entered the patient data. XH Z was a major contributor in writing the manuscript. All authors have read and approved the final manuscript.

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Not applicable.

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Figures

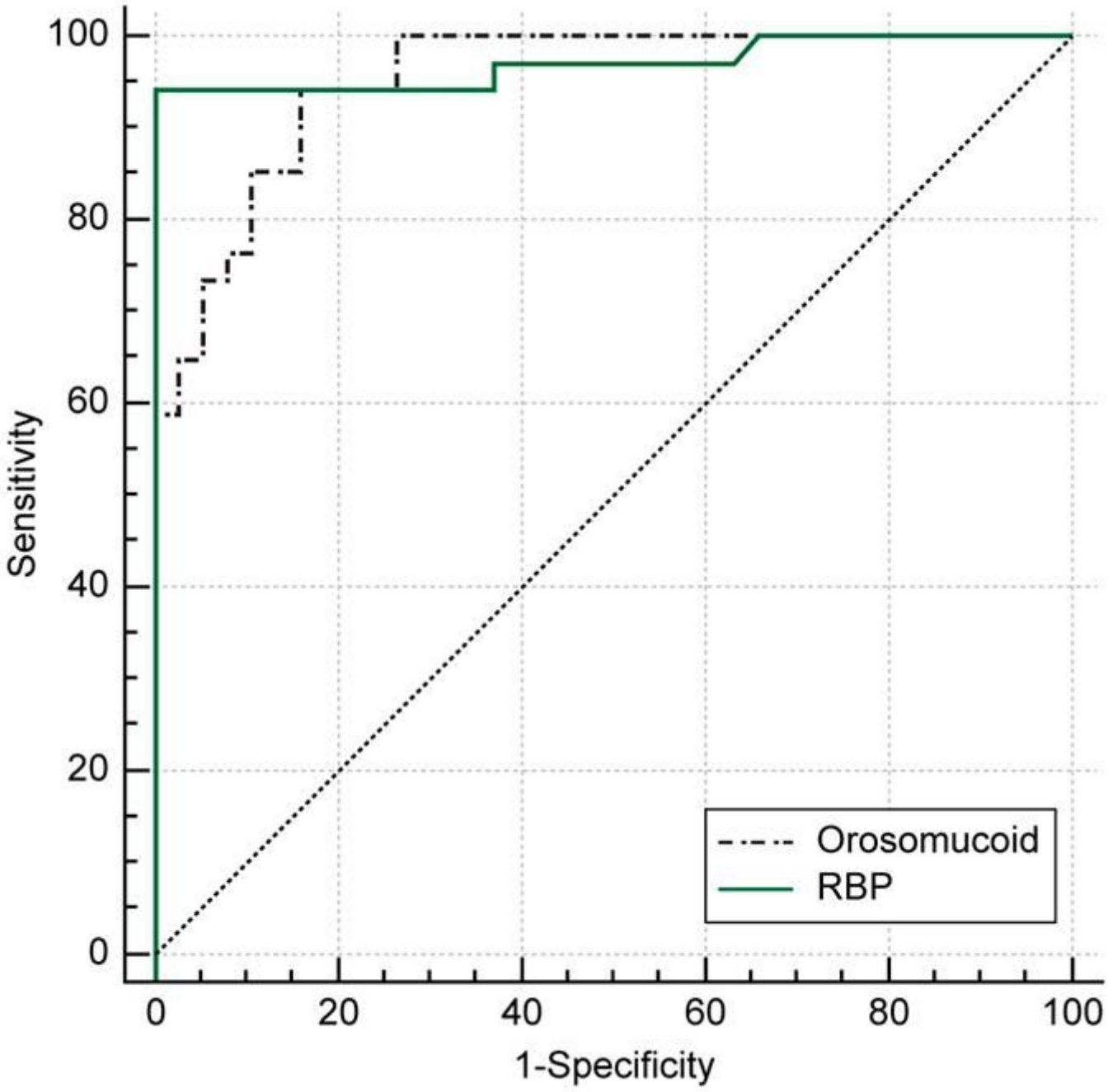

Figure 1

Receiver operating characteristic curve of two indicators for predicting diabetic nephropathy RBP: retinol binding protein