

Original Article

Serum copeptin as a new biomarker in the early diagnosis of decline in renal function of type 2 diabetes mellitus patients

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Abstract: Objective: This study aimed to investigate the correlation between serum copeptin and glomerular filtration rate (GFR) in type 2 diabetes mellitus (T2DM) patients and to investigate the role of serum copeptin in the diagnosis of early DN in T2DM patients. Methods: 120 T2DM inpatients were recruited and divided into 2 groups according to 24-h urine albumin excretion (UAE): normal UAE group (UAE<30 mg/24 h) and microalbuminuria group (30 mg/24 h≤UAE≤300 mg/24 h). Results: Decline in GFR was found in 6.1% of patients in normal UAE group and 26.4% in microalbuminuria group. However, serum copeptin was comparable between two groups. Serum copeptin was negatively related to GFR ($r=-0.586$, $P<0.001$). Multivariate logistic regression analysis showed, after adjustment for age and gender, the OR of copeptin, 24-h UAE was 1.234 (95% CI: 1.003-1.456) ($P<0.05$) and 1.068 (95% CI: 1.005-1.187) ($P<0.05$), respectively. Univariate analysis of ROC showed the sensitivity of copeptin and 24-h UAE was 78.9% and 63.2%, respectively and the specificity was 88.9% and 89.7%, respectively in the diagnosis of DN, but the area under ROC of copeptin in combination with 24-h UAE was 0.90 (95% CI: 0.82-0.99) with the sensitivity of 80.9% and specificity of 91.1%. Conclusion: Serum copeptin is an independent risk factor of decline in renal function of T2DM patients. Copeptin in combination with 24-h UAE are helpful for the early diagnosis of DN. The causative relationship between serum copeptin and GFR is required to be further studied in long-term follow up.

Keywords: Copeptin, glomerular filtration rate, urine albumin excretion

Introduction

Studies have shown that about 40% of type 1 diabetes mellitus (T1DM) patients [1] and approximate 25% of type 2 diabetes mellitus (T2DM) patients [2] may develop diabetic nephropathy (DN). Currently, the increase in urine albumin excretion (UAE) has been regarded a manifestation of early DN. However, UKPDS found that about 51% of T2DM patients developed chronic kidney dysfunction in the absence of albuminuria [3]. Clinically, the glomerular filtration rate (GFR) cannot be directly measured. It is accepted that the clearance of exogenous radionuclide is more accurately reflect GFR, but this detection is highly expensive, which significantly limits its wide application in clinical practice and large-scale survey. Thus, it is imperative to identify new biomark-

ers for the rapid and accurate diagnosis of early DN. Previous studies showed pituitary vasopressin plays important roles in the regulation of osmotic pressure, glucose homeostasis and inflammation. Copeptin is the C terminal of stable pro-vasopressin and may serve as a potent biomarker of metabolic diseases and DM [4, 5]. In recent years, studies also find that copeptin may increase the urine protein [6] and predict the outcomes of adverse events of heart and kidney [7, 8]. Thus, we hypothesized that copeptin may be a sensitive biomarker that can predict the change in kidney function and has clinical importance in the early diagnosis and progression evaluation of DN. In the present study, the relationship between serum copeptin and GFR determined by renal dynamic imaging was evaluated in DM patients, and the association of copeptin and change in kidney function

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in T2DM patients and the factors affecting the kidney function of DM patients were also explored, aiming to identify relevant risk factors.

Materials and methods

Patients

Hospitalized patients with T2DM were recruited from the Department of Endocrinology of our hospital between March 2014 and February 2015, and T2DM was diagnosed according to the diagnostic criteria of WHO. GFR determined by renal dynamic imaging, serum copeptin, serum creatinine (Scr) and 24-h UAE were measured. T1DM, secondary DM, immune diseases, hepatitis, pregnancy, urinary tract infection, acute complications of DM (diabetic ketoacidosis and nonketotic hyperosmolar coma), other chronic renal diseases, thyroid dysfunction and uncontrolled hypertension, coronary heart disease, myocardial infarction and peripheral vascular diseases were excluded from these patients. According to the 24-UAE, patients were divided into 2 groups: normal UAE group (UAE<30 mg/24 h) and microalbuminuria group (30 mg/24 h≤UAE≤300 mg/24 h).

Patients' characteristics at baseline

Following information was collected from patients recruited: gender, age, course of disease, history of hypertension, use of ACEI and ARB, blood pressure and body mass index (BMI).

Clinical parameters

On the second day following admission, venous blood was collected from patients fasted for 8-12 h and fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), Scr, uric acid (UA), cystatin (Cys-c) and fasting C-peptide (FC-P) were measured. FC-P was used to estimate the Homeostasis Model Assessment (HOMA) because different protocols were used among these patients [9]: $HOMA-IR=1.5+FPG \times FC-P/2800$ and $HOMA-islet=0.27 \times FC-P/(FPG-3.5)$.

Between the second day and the third day, 24-h urine was collected for the detection of UAE.

Diabetic retinopathy was evaluated by an experienced ophthalmologist.

Measurement of serum copeptin

On admission, venous blood (1 ml) was collected into a tube from patients fasted for 12 h and incubated at 37°C for 2 h. Then, centrifugation was done at 3000 rpm/min for 10 min. The serum was harvested and stored at -80°C for the detection of copeptin. Serum copeptin was detected by ELISA with copeptin assay kit (Nanjing Kengen Biotech Co., Ltd).

Detection of GFR by dynamic radionuclide renal imaging

Particle enhanced turbidimetric assay (PETIA) was employed to detect GFR via renal dynamic imaging (SPECT/CT with InfiniaVCHawkeyeAC dual probes; GE, USA). ⁹⁹Tcm-DTPA (purity: >95%) was provided by Guangdong Xi'ai Hospital. At 30 min before renal dynamic imaging, patients drunk 300 mL of water, followed by bolus injection of ⁹⁹Tcm-DTPA and subsequent renal dynamic imaging by SPECT. Gate's method was used to calculate GFR of unilateral and bilateral kidney, which was then adjusted for body surface with Stevenson formula as follow: $S=0.0061 \times \text{height (cm)}+0.0128 \times \text{body weight (kg)}-0.1529$.

On the basis of GFR in NKF guideline [10], chronic renal disease is divided into 5 stages: stage 1: GFR>90 mL/min/1.73 m²; stage 2: 60 mL/min/1.73 m²<GFR<90 mL/min/1.73 m²; stage 3: 30 mL/min/1.73 m²≤GFR<60 mL/min/1.73 m²; stage 4: 15 mL/min/1.73 m²≤GFR<30 mL/min/1.73 m² and stage 5: GFR<15 mL/min/1.73 m². Decline in renal function was defined at GFR of <60 mL/min/1.73 m².

Statistical analysis

Statistical analysis was performed with SPSS version 13.0. Quantitative data with normal distribution were expressed as mean ± standard deviation and compared with independent *t* test between groups and one way analysis of variance followed by LSD method among groups. Data with abnormal distribution were expressed as median (range) and compared with rank sum test. Association was evaluated with Pearson correlation analysis for data with normal distribution and Spearman rank corre-

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Table 1. Characteristics of study participants

Variables	UAE (mg/24 h)		P-value
	<30 (n=67)	30-300 (n=53)	
Male, n (%)	35 (52.2)	30 (56.6)	0.357
Age (years)	56.92±14.07	60.06±9.61	0.986
Use of ACE inhibitors or ARB, n (%)	18 (27.3)	17 (32.1)	0.635
History of hypertension, n (%)	30 (44.8)	19 (35.8)	0.192
Course of diabetes, n (%)	6.79±6.57	9.52±7.14	0.165
DR, n (%)	12 (17.9)	11 (20.8)	0.275
BMI (kg/m ²)	25.13±2.86	25.85±2.58	0.919
SBP (mmHg)	132.12±18.45	158.21±20.14	0.000
DBP (mmHg)	81.27±13.34	91.24±11.12	0.000
FPG (mg/dl)	207.44±113.04	241.92±68.4	0.013
HbA1c (%)	9.45 (5.7, 17.0)	9.98 (6.0, 17.6)	0.225
FC-P (ng/dl)	1.39 (0.03, 4.78)	2.52 (1.37, 3.80)	0.000
HOMA-IR	1.67 (1.52, 2.87)	1.94 (1.60, 2.14)	0.001
HOMA-islet	2.11 (0.01, 13.03)	3.41 (0.66, 6.45)	0.056
TC (mmol/l)	4.90±1.26	5.70±1.43	0.000
TG (mmol/l)	1.36 (0.26, 9.70)	2.35 (0.67, 9.19)	0.000
LDL-C (mmol/l)	2.83±0.91	3.39±1.0	0.184
HDL-C (mmol/l)	1.21±0.47	0.99±0.25	0.000
SUA (mg/dl)	2.87±1.88	3.56±1.44	0.004
Cys-c (mg/L)	0.92 (0.48, 22.69)	1.58 (0.51, 2.47)	0.000
BUN (mmol/l)	6.22 (3.43, 9.44)	8.90 (5.98, 11.33)	0.000
UAE (mg/ml)	6.0 (1.0, 21.2)	55.7 (30, 273)	0.000
Serum creatinine (mg/dl)	0.79±0.36	0.86±0.43	0.057
GFR (mL/min/1.73 m ²)	96.87±28.70	79.59±28.81	0.000
1 >90	40 (59.7)	19 (35.8)	
2 60-90	23 (34.3)	20 (37.7)	
3 30-60	4 (6.0)	14 (26.4)	
4 <30	NA	NA	
Copeptin (ng/L)	802.05±383.65	952.80±352.89	0.162

Footnotes: data are presented as means (±SD) or number (%). Means and proportions were compared by ANOVA and Fisher's exact test, respectively. P values tested the overall difference among UAE Classifications. Definitions of hypertension, diabetes were described in Methods. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; FPG, fasting plasma glucose; TG, triglycerides; TC, total cholesterol; SUA, serum uric acid; Cys-c, cystatin; BUN: blood urea nitrogen; HbA1c, glycated hemoglobin; FC-P, fasting C-peptide; GFR, glomerular filtration rate; UAE, 24-h urine albumin excretion; HOMA, homeostasis model assessment; International system of units (SI) conversion: plasma glucose 1 mg/dl=1/18 mmol/l; SUA 1 mg/dl=59.5 mmol/l; Serum creatinine, 1 mg/dL=88.41 umol/L; HOMA-IR=1.5+FPG×FC-P/2800; HOMA-islet=0.27×FC-P/FPG-3.5.

lation analysis for those with abnormal distribution. A value of P<0.05 was considered statistically significant. Binary logistic regression was employed for multivariate analysis of risk factors causing decline in renal function. In addition, receiver operating characteristic curve (ROC) was delineated, followed by stepwise regression analysis of the role of copeptin and/or 24-h UAE in the differential diagnosis of decline in renal function.

Results

General information

Of 120 patients recruited, the mean age was 57.52±12.81 years, and there were 65 males and 55 females. In addition, 67 patients (55.5%) were in normal UAE group and 53 (44.5%) had microalbuminuria. Among 120 patients, the mean GFR was 89.75±29.81 mL/min.1.73 m². In normal UAE group and microal-

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Table 2. Correlation between copeptin and clinical parameters in T2DM patients

Variables	Copeptin (mg/mL)	
	r	P-value
Age (years)	0.179	0.019
Course of diabetes (years)	0.093	0.392
BMI (kg/m ²)	-0.021	0.548
SBP (mmHg)	0.247	0.000
DBP (mmHg)	0.257	0.000
FPG (mg/dl)	0.100	0.709
HbA1c (%)	0.186	0.005
FC-P (ng/dl)	0.138	0.03
TC (mmol/l)	0.221	0.415
TG (mmol/l)	0.157	0.019
LDL-C (mmol/l)	0.088	0.903
HDL-C (mmol/l)	-0.120	0.053
SUA (mg/dl)	0.267	0.001
Cys-c	0.399	0.000
BUN	0.234	0.000
Scr	0.274	0.000
UAE	0.171	0.008
GFR	-0.586	0.000
HOMA-IR	0.150	0.018
HOMA-islet	0.017	0.664

Footnotes: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; FC-P, Fasting C-peptide; TC, Total cholesterol; TG, Triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; SUA, serum uric acid; Cys-c, cystatin; BUN: blood urea nitrogen; Scr: serum creatinine; UAE, 24-h urine albumin excretion; GFR, glomerular filtration rate; International system of units (SI) conversion: plasma glucose 1 mg/dl=1/18 mmol/l; SUA 1 mg/dl=59.5 mmol/l; HOMA, homeostasis model assessment; HOMA-IR=1.5+FBG×FC-P/2800; HOMA-islet≤UAE=0.27×FC-P/FPG-3.5.

buminuria group, the proportion of patients with GFR reduction was 6.1% (4/67) and 26.4% (14/53), respectively, and a total of 18 patients (15%) had GFR reduction. There were no marked differences in age and gender between groups. In microalbuminuria group, the mean GFR was lower than that in normal UAE group. Significant differences were observed in the SBP, DBP, FPG, Cys-c, BUN, SUA, TC, TG, HDL-C, HOMA-IR and 24-h UAE between two groups ($P<0.05$). Serum copeptin, course of DM, diabetic retinopathy, HbA1C, use of ACEI and ARB, history of hypertension, DR, BMI, HOMA-islet, LDL-C and Scr were comparable between groups ($P>0.05$) (Table 1).

Factors related to serum copeptin in DM patients

Serum copeptin was positively associated with age, SBP, DBP, HbA1c, TG, SUA, Bun, Cys-c, Scr, 24-h UAE and HOMA-IR, but negatively related to GFR (Table 2).

Factors affecting the decline in renal function

For the regression analysis of 120 patients with T2DM, GFR served as a dependent variable and risk factors of renal diseases (Table 1) as independent variables. Multivariate logistic regression analysis was performed after adjustment for age and gender, and results showed copeptin, 24-h UAE and insulin resistance index were independent risk factors (Table 3).

Role of Copeptin and/or 24-h UAE in the diagnosis of renal function decline by ROC analysis

Of 120 patients with T2DM, serum copeptin and 24-h UAE were used to delineate ROC, and the sensitivity and specificity were analyzed with maximal Youden index. ROC analysis of single variable showed the sensitivity of copeptin and 24-h UAE was 78.9% and 63.2%, respectively, and the specificity was 88.9% and 89.7%, respectively, in the diagnosis of renal function decline in T2DM patients. Stepwise logistic regression analysis was used to establish a model for fit ROC (Figure 1). Results showed the area under ROC (AUC) of copeptin and 24-h UAE was 0.88 (95% CI: 0.78-0.98) and 0.82 (95% CI: 0.70-0.95), respectively. However, the AUC, sensitivity and specificity of copeptin in combination with 24-h UAE were 0.90 (95% CI: 0.82-0.99), 80.9% and 91.1%, respectively.

Discussion

The increase in UAE has been regarded the earliest clinical manifestation of DN. In as early as 1992, LANE et al [11] reported T1DM patients with normal albumin excretion and reduced creatinine clearance, which was also confirmed in other studies. Moreover, this phenomenon is also found in T2DM patients. In recent years, there is evidence showing that the use of ACEI or ARB may reduce the incidence of newly onset microalbuminuria in T2DM patients [12]. However, MACLSAAC et al [13] excluded patients who were treated with these drugs

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Table 3. Multiple logistic regression analysis of risk factors for mild reduced GFR

Independent variable	Multivariable adjusted*	
	OR (95% CI)	P-value
Gender	1.574 (0.034-72.723)	0.816
Age	1.016 (0.846-1.221)	0.863
Course of diabetes	1.076 (0.774-1.495)	0.664
SBP	1.126 (0.978-1.298)	0.100
BMI	0.526 (0.270-1.026)	0.059
HbA1c	0.661 (0.381-1.290)	0.142
SUA	1.019 (0.993-1.209)	0.101
HOMA-IR	0.020 (0.01-0.104)	0.018
UAE	1.068 (1.005-1.187)	0.033
Copeptin	1.234 (1.003-1.456)	0.012

Footnotes: CI, confidence interval; OR, odds ratio; SBP, systolic blood pressure; BMI, body mass index; HbA1c, glycated hemoglobin; SUA, serum uric acid; HOMA, homeostasis model assessment; $HOMA-IR = 1.5 + FPG \times FC - P / 2800$; UAE, 24-h urine albumin excretion. *Adjusted for age and gender, history of hypertension, course of diabetes, use of ACE inhibitors or ARB.

before study, and their results showed 23% of T2DM patients had GFR of <60 mL/min/ 1.73 m² in the absence of microalbuminuria. Of 120 patients in the present study, 18 (15%) had GFR <60 mL/min/ 1.73 m², and the proportion of patients with GFR reduction was 6.0% and 26.4% in normal UAE group and microalbuminuria group, respectively. This suggests that some T2DM patients have developed decline in renal function although UAE is normal.

Although the detection of inulin clearance and radionuclide method may accurately reflect GFR, it is complex and highly expensive, significantly limiting its wide application in clinical practice. Currently, Scr and other parameters are used to reflect the renal injury. However, studies have shown that Scr is influenced by some factors including race, age, gender, muscle mass and diet, and especially in the elderly, Scr may be still normal when the GFR is <50 mL/min [14]. Thus, available biomarkers cannot meet the clinical requirements, and it is imperative to identify new biomarkers of DN, which has been a focus in current studies on DN.

In recent years, studies [4, 5, 15] reveal that arginine vasopressin (AVP) released by the pituitary following chronic psychological stress may induce insulin resistance, obesity and metabolic syndrome. AVP is a bioactive peptide pro-

duced by the hypothalamus [16, 17] and able to regulate the osmotic pressure, maintain the hemodynamic stability and regulate the central nervous system. AVP has been one of important stress hormones [18]. AVP is released in pulses and highly unstable *in vivo*, and has a short half-life (10-20 min). Thus, it is difficult to detect AVP *in vitro*, which significantly limits the clinical application of AVP [19]. However, copeptin is a fragment of C terminal of AVP, composed of 39 amino acids and released with an equimolar amount to AVP [20]. In addition, copeptin is stable *in vitro* and easy to detect. Thus, the measurement of copeptin may replace the direction detection of AVP.

In the present study, although serum copeptin was comparable between normal UAE group and microalbuminuria, serum copeptin was positively related to 24-h UAE (microalbuminuria) and negatively associated with GFR. After adjustment for age and gender, these associations were still present, but the specific mechanism is unclear. In recent years, a study on healthy subjects [21] also revealed that copeptin was associated with proteinuria. However, in a study on polycystic kidney disease [22], high serum copeptin was found to be related to poor renal function, decline in renal blood flow, glomerular hypertrophy and proteinuria. These were ascribed to the activation of V2 receptor in the distal tubules and collecting ducts and subsequently increase in cAMP in the epithelial cells. Vesicles containing water channel and locating around the luminal membrane are inlaid into the luminal membrane which increases the water channel, the water permeability and the vesicular growth. In addition, there is evidence showing that desmopressin acetate is able to increase the excretion of protein in the urine in humans and rodents [6], but V2 receptor antagonist inhibits the UAE in diabetic rats [23], suggesting that V2 receptor activation causes a decline in renal function.

Velho et al investigated 3101 patients with T2DM and proteinuria from DHABHYCAR study [8] and patients were grouped according to the tertile of serum copeptin at baseline. Their results showed the incidence of renal events was 1.06% in T1 group, 1.45% in T2 group and 4.84% in T3 group. For patients with heavy proteinuria at baseline, the incidence of renal

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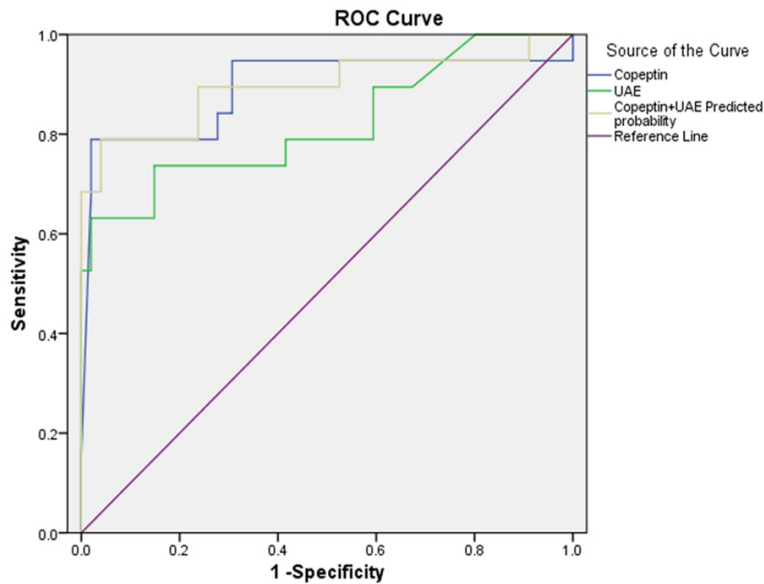


Figure 1. ROC curve of renal function decline with Copeptin and/or UAE in T2DM patients.

events was 2.43% in T1 group, 5.11% in T2 group and 11.81% in T3 group. The HR of serum copeptin as a risk factor of renal events was 4.79. Study shows serum copeptin is helpful for the identification of chronic DN patients with high risk for a decline in renal function. However, in the present study, serum copeptin was positively related to the age, SBP, DBP, HbA1c, TG, SUA, BUN, Cys-c, Scr, and 24-h microalbuminuria which are risk factors of DN, but negatively associated with GFR determined by dynamic radionuclide renal imaging. In the multiple regression analysis, serum copeptin was still an independent risk factor of renal function decline after adjustment for age and gender. Thus, serum copeptin not only predicts the outcome of renal adverse events, but may serve as a sensitive indicator of early decline in renal function. Analysis with ROC showed, when the GFR was <60 mL/min/1.73 m² in T2DM patients, the diagnostic value of serum copeptin (AUC=0.88) was better than that of 24-h UAE (AUC=0.82), and the optimal cut-off value of serum copeptin was 1197.10 ng/L. Moreover, the diagnostic value of serum copeptin in combination with 24-h UAE increased significantly (AUC=0.90). Thus, we speculate that serum copeptin in combination with 24-h UAE may serve as an effective tool in the early diagnosis of DN in clinical practice.

There were some differences in the present study as compared to previous studies: (1) this

study focused on the role of copeptin in the early diagnosis of DN. Thus, subjects with normal UAE and microalbuminuria were recruited, and renal adverse events were not evaluated. (2) GFR was measured by dynamic radionuclide renal imaging, not by estimation as in previous studies. Our results showed GFR determined by dynamic radionuclide renal imaging is still a gold standard in the evaluation of renal diseases. There are disadvantages in the formulas used for the estimation of GFR, and no formula for the estimation of GFR is completely suitable for Chinese patients.

There were still limitations in this study. Firstly, the sample size was relatively small, and our findings are required to be confirmed in future studies with a large sample size. Secondly, this was a cross-sectional study, and failed to confirm the causative relationship between copeptin and GFR. Thus, studies with long-term follow up are required to validate whether there is causative relationship between them. Finally, patients recruited were hospitalized, and had poor glucose control and concomitant hypertension. Thus, we could not completely exclude the influence of hypertension in the kidney.

Taken together, serum copeptin is an independent risk factor of decline in renal function of T2DM patients. Serum copeptin in combination with 24-h microalbuminuria are beneficial for the early diagnosis of DN. The causative relationship between serum copeptin and GFR is warranted to be confirmed in studies with long-term follow up.

Disclosure of conflict of interest

None.

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