Observational Study

Risk factors and urinary biomarkers of non-albuminuric and albuminuric chronic kidney disease in patients with type 2 diabetes

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Abstract

BACKGROUND

A number of recent studies indicate a transformation in the natural course of chronic kidney disease (CKD) in type 2 diabetes (T2D) patients: an increasing prevalence of declined renal function without proceeding to the accompanying elevation of albuminuria. It has been suggested that albuminuric and non-albuminuric CKD patterns could be different in their phenotypes and pathogenic mechanisms.

AIM

To identify the risk factors and biomarkers of albuminuric and non-albuminuric patterns of CKD in patients with T2D.

METHODS

Three hundred sixty patients with T2D duration ≥ 10 years were included in this observational cross-sectional study. The associations of a panel of demographic and clinical characteristics, complications, comorbidities, and metabolic and hematology parameters with albuminuric and non-albuminuric CKD patterns were analyzed. The urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis, was determined by ELISA in comparison with healthy controls.

RESULTS

Non-albuminuric CKD was associated with age ≥ 65 years (P = 0.0001), female
INTRODUCTION

The increasing prevalence of diabetes around the world and changes in diabetes management have transformed the epidemiology of chronic kidney disease (CKD) in recent years. In many countries, including the United States, diabetes is responsible for over 40% of new cases of end-stage renal disease (ESRD), surpassing other causes to become the leading driver of the renal impairment[1]. Despite the fact that the prevalence of CKD among adult patients with diabetes remains stably high, a transformation in its natural course has been recorded. According to the classical paradigm, albuminuria is an early indicator of diabetic kidney disease. A number of recent studies have documented an increasing proportion of diabetic patients in whom a reduction in renal function develops without a preceding or concomitant increase in albuminuria[1]. This tendency is most evident in type 2 diabetes (T2D); the proportion of the non-albuminuric CKD (NA-CKD) pattern in this type of disease prevalence of CKD among adult patients with diabetes remains stably high, a transformation in its natural course has been recorded. According to the classical paradigm, albuminuria is an early indicator of diabetic kidney disease. A number of recent studies have documented an increasing proportion of diabetic patients in whom a reduction in renal function develops without a preceding or concomitant increase in albuminuria[1]. This tendency is most evident in type 2 diabetes (T2D); the proportion of the non-albuminuric CKD (NA-CKD) pattern in this type of disease currently ranges from 40% to 70%[1].

The causes for the shift in the natural course of diabetic kidney disease are not fully understood. Among others, the wide use of renin-angiotensin system blockers; advances in antihyperglycemic, antihypertensive, and hypolipidemic therapy; and sex (P = 0.04), diabetes duration ≥ 15 years (P = 0.0009), and the use of diuretics (P = 0.0005). Male sex (P = 0.01), smoking (P = 0.01), waist-to-hip ratio > 1.0 (P = 0.01) and hemoglobin A1c (HbA1c) > 8.0% (P = 0.005) were risk factors for elevated albuminuria not accompanied by a decrease in estimated glomerular filtration rate (eGFR). Duration of diabetes ≥ 15 years and the use of calcium channel blockers were risk factors for albuminuria with decreased eGFR (both P = 0.01). In multivariate logistic regression analysis, age, HbA1c, female sex and diuretics were significant predictors for reduced eGFR, while waist-to-hip ratio, HbA1c and male sex were associated with elevated urinary albumin-to-creatinine ratio (UACR). Excretion of nephrin and podocin was increased in patients with albuminuria, regardless of decline in renal function (P < 0.001), correlating positively with UACR. The urinary excretion of WFDC-2 was markedly higher in men than in women (P < 0.000001). Men with T2D demonstrated increased WFDC-2 levels independently of the CKD pattern (all P < 0.05). In T2D women, WFDC-2 excretion was increased in those with reduced renal function (P ≤ 0.01), correlating negatively with eGFR.

CONCLUSION

The data provide further evidence that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

Key words: Diabetes mellitus; Chronic kidney disease; Albuminuria; Glomerular filtration rate; Podocytes; Risk factors; Biomarkers

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Core tip: In this study, we demonstrate the differences in clinical and laboratory characteristics between albuminuric and non-albuminuric phenotypes of chronic kidney disease (CKD) in patients with long-term type 2 diabetes. Different risk factors are found for three different CKD phenotypes. We also show the diversity in the urinary excretion of nephrin and podocin, two slit diaphragm proteins, and of WAP-four-disulfide core domain protein 2, a tubulointerstitial fibrosis marker, between different CKD phenotypes. The results further support the notion that albuminuric and non-albuminuric CKD phenotypes are different in their pathophysiology and clinical characteristics.

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smoking cessation are discussed\(^2\text{-}^3\). New classes of antihyperglycemic agents, including glucagon-like peptide-1 (GLP-1) analogs, dipeptidylpeptidase-4 (DPP-4) inhibitors, and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, have demonstrated a distinct antialbuminuric effect in clinical trials\(^6\text{-}^9\). Accordingly, the growing use of these agents in clinical practice may cause a reduction in the prevalence of albuminuria among diabetic patients.

Presently, little is known about the clinical phenotypes and pathophysiology of albuminuric and NA-CKD patterns in diabetes. A growing body of evidence indicates that these patterns demonstrate significant differences in natural course and outcomes. Even if a more favorable situation in terms of the risk of ESRD, NA-CKD is clearly associated with cardiovascular disease and its risk factors\(^4\). Accordingly, the non-albuminuric phenotype might be related to macroangiopathy instead of microangiopathy and/or be the consequence of repeated and/or unresolved episodes of acute kidney injury, even of a mild degree\(^1\text{-}^2\). When comparing renal biopsy findings associated with normo-, micro-, or macroalbuminuria in T2D patients with glomerular filtration rate (GFR) less than 60 mL/min/1.73 m\(^2\), typical glomerular changes were revealed mostly in patients with elevated albuminuria. In those with NA-CKD, predominant interstitial and vascular changes were more frequent findings\(^1\text{-}^2\). It was speculated that non-albuminuric renal impairment represents a different pathway to the loss of renal function compared to albuminuric one.

Podocyte injury has been identified as a pivotal event resulting in proteinuric kidney disease, glomerulosclerosis, and loss of renal function\(^1\text{-}^2\). During filtration, plasma passes through a sieve consisting of a fenestrated endothelium and a broad basement membrane before it reaches the most unique part, the slit diaphragm, a specialized type of intercellular junction that connects neighboring podocyte foot processes. When podocytes become stressed, irrespective of the causative stimulus, they undergo foot process effacement and loss of slit diaphragms – two key steps leading to proteinuria\(^3\). It was demonstrated that not only proteinuria but also tubulointerstitial lesions should be assessed to predict rapid GFR decline in patients with T2D who have overt proteinuria\(^3\). Moreover, it was reported that interstitial fibrosis, tubular atrophy and interstitial inflammation, but not glomerular lesions, are significant predictors for renal prognosis in T2D patients with overt proteinuria\(^4\). In a recent study, interstitial fibrosis and tubular atrophy score, as well as glomerular basement membrane thickness, were independent predictors for renal replacement therapy initiation in T2D patients\(^4\). Taking into account the results of morphological investigations, the assessment of urinary markers of podocyte and interstitial involvement may provide further information on the development of different CKD phenotypes. This study aimed to identify the risk factors, as well as the markers of podocyte and interstitial involvement, in albuminuric and NA-CKD in patients with T2D.

**MATERIALS AND METHODS**

**Ethical issues**

The study protocol was approved by the Ethical Committee of the Research Institute of Clinical and Experimental Lymphology – branch of the Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences. All patients provided their written informed consent prior to the inclusion.

**Design**

The design of this observational, single-center, cross-sectional study is presented in Figure 1. Adult men and women with T2D duration of at least 10 years from the date of diagnosis were included. Non-diabetic CKD, ESRD, urinary tract infection, ketoacidosis or hyperosmolar state at the time of the survey, treatment with DPP-4 inhibitors, GLP-1 receptor agonists and/or SGLT-2 inhibitors for three months prior to inclusion, malignant neoplasms, inflammatory or autoimmune diseases in the medical history, and a high-protein diet acted as exclusion criteria.

**Subjects**

Five hundred six potentially eligible T2D patients who met the inclusion criteria were selected. After evaluation for exclusion criteria, 360 patients, 100 men and 260 women, from 43 to 88 years of age (median 66 years), were included in the analysis. Twenty individuals who had no history of diabetes, obesity or cardiovascular disease, including 13 women and 7 men, from 50 to 74 years of age (median 62.5 years), acted as controls in the study of urinary biomarkers.

**Methods**
The study was designed as an observational, single-center, cross-sectional study. Adult men and women with type 2 diabetes (T2D) duration of at least 10 years from the date of diagnosis were included (n = 506). After evaluation for exclusion criteria, 360 patients were included in the analysis. Patients were divided into four groups according to their estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR) levels. Individuals with eGFR ≥ 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were recorded as patients without chronic kidney disease (CKD) signs (CKD– group). Those with eGFR < 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were assigned to the non-albuminuric chronic kidney disease group. Patients with eGFR ≥ 60 mL/min × 1.73 m² and UACR ≥ 3.0 mg/mmol comprised the albuminuric CKD group (A-CKD+). All patients underwent clinical examination, which included an evaluation of diabetes control and in-depth screening/monitoring of complications and comorbidities. The set of clinical risk factors was estimated for each CKD pattern. Urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2, a marker of tubulointerstitial fibrosis, was assessed in T2D patients and the control group (20 subjects without a history of diabetes, obesity or cardiovascular disease). CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD–: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.

All patients underwent clinical examination, which included an evaluation of diabetes control and in-depth screening/monitoring of complications. Routine laboratory measurements, including glycated hemoglobin A1c (HbA1c), total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides and uric acid, were performed on AU480 Chemical Analyzer (Beckman Coulter, United States) with commercially available cartridges. The HbA1c levels were measured by turbidimetric immunoinhibition method. A kinetic enzymatic method was applied for determination of the levels of lipids and uric acid. Three fasting and three 2-h postprandial blood glucose values were obtained daily from each patient in three-day series. The measurements were performed by One Touch Verio® (Johnson and Johnson/Lifescan, United States) glucose meter. A complete blood count was performed on a hematology analyzer (Analyticon Biotechnologies AG, Germany). Concentrations of fibrinogen, soluble fibrin monomer complex (SFMC) and D-dimer were evaluated on the hemostasis analyzer system (Instrumentation Laboratory, United States).

The levels of creatinine in serum and urine were determined on AU480 Chemical Analyzer (Beckman Coulter, United States) by modified kinetic Jaffé’s method. The estimated GFR (eGFR) was calculated by the CKD-EPI formula (2009). Urinary albumin was determined in three morning urine samples by immunoturbidimetry on AU480 Chemical Analyzer (Beckman Coulter, United States) in accordance with the manufacturer’s instructions. The mean albumin concentration (mg) was adjusted to

**Figure 1 The design of the study.** The study was designed as an observational, single-center, cross-sectional study. Adult men and women with type 2 diabetes (T2D) duration of at least 10 years from the date of diagnosis were included (n = 506). After evaluation for exclusion criteria, 360 patients were included in the analysis. Patients were divided into four groups according to their estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (UACR) levels. Individuals with eGFR ≥ 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were recorded as patients without chronic kidney disease (CKD) signs (CKD– group). Those with eGFR < 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were assigned to the non-albuminuric chronic kidney disease group. Patients with eGFR ≥ 60 mL/min × 1.73 m² and UACR ≥ 3.0 mg/mmol comprised the albuminuric CKD group (A-CKD+). All patients underwent clinical examination, which included an evaluation of diabetes control and in-depth screening/monitoring of complications and comorbidities. The set of clinical risk factors was estimated for each CKD pattern. Urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2, a marker of tubulointerstitial fibrosis, was assessed in T2D patients and the control group (20 subjects without a history of diabetes, obesity or cardiovascular disease). CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; NA-CKD: Non-albuminuric chronic kidney disease; T2D: Type 2 diabetes; UACR: Urinary albumin-to-creatinine ratio; WFDC-2: WAP-four-disulfide core domain protein 2; CKD–: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD–: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.
the excreted creatinine (mmol) and is expressed as the urinary albumin/creatinine ratio (UACR). Urinary excretion of total protein was assessed by colorimetric method with pyrogallol red-molybdate complex on AU480 Chemical Analyzer (Beckman Coulter, United States).

In this study, we assessed the urinary excretion of nephrin and podocin, two podocyte-specific markers, and of WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis. Both nephrin and podocin are expressed on the surface of podocytes, acting as components of the slit diaphragm complex; accordingly, these molecules are used as markers of dysfunction and injury of podocytes. The increase in urinary excretion of nephrin and podocin in patients with diabetes was reported in a number of studies. WFDC-2, also known as human epididymal protein-4 (HE-4), is expressed by myofibroblasts. Focal and low expression of WFDC-2 is found in the distal convoluted tubule of the kidney. It was shown that WFDC-2 suppressed the activity of multiple proteases, including serine proteases and matrix metalloproteinases, and specifically inhibited their capacity to degrade type I collagen. Recently, serum WFDC-2 has been validated as a clinical marker of renal fibrosis.

The morning urine samples for the biomarker assay were centrifuged, and the supernatants were separated and stored at -80 °C until analysis. Repeated freeze-thaw cycles were avoided. The concentrations of nephrin, podocin and WFDC-2 in the urine were assessed by ELISA using commercially available kits (Cloud-Clone Corp., United States, catalog/serial No. SEA937Hu/5A788AAD51, SEA938Hu/6E430916C8 and SEA241Hu/8D970EE435, respectively), in accordance with the manufacturer’s instructions. The results were adjusted to urinary creatinine and compared to the control.

In-depth screening/monitoring of diabetic complications and associated conditions was performed in all patients. Diabetic retinopathy was diagnosed by ophthalmologist with a comprehensive dilated eye examination. Coronary artery disease was defined as myocardial infarction, unstable angina, coronary revascularization procedure, or transient myocardial ischemia in medical history, and/or abnormal result of exercise ECG testing or stress echocardiography. Chronic heart failure was assessed by New York Heart Association functional classification taking into account the limitation to physical activity, the results of physical examination and echocardiography. Carotid atherosclerosis and peripheral artery disease was verified by duplex ultrasound.

**Statistical analysis**
The statistical software package Statistics 12.0 (Dell, United States) was used to analyze the results. Quantitative data are presented as medians (lower quartiles; upper quartiles). Frequencies are presented as percentages (%). The normal distribution was determined by the Kolmogorov–Smirnov test. Because most of the quantitative were not distributed normally, non-parametric multiple comparisons of mean ranks were used to assess the statistical significance of differences between groups by continuous characteristics. The statistical significance of differences in discrete parameters between groups was analyzed using the χ² test. A difference was defined as significant if the P value was less than 0.05. Spearman rank correlation analysis was applied to test the association between variables. To assess the contribution of the investigated parameters to declining eGFR and development of albuminuria, a multiple logistic regression was used. The contribution of the factor was defined as significant if the standard deviation of the coefficient β did not exceed the coefficient β and the P value was less than 0.05. To assess the significance of the studied factors, the odds ratio, 95% confidence interval (CI), and P value were calculated using MedCalc 18.11.6 (MedCalc Software, Belgium). The influence of the factor was determined to be significant when the boundaries of the 95%CI were located on the same side of 1.0 and the P value was less than 0.05.

**RESULTS**

**Clinical and laboratory characteristics of T2D patients with different CKD patterns**
Patients were divided into four groups according to their eGFR and UACR levels. The individuals with eGFR ≥ 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were recorded as patients without CKD signs (CKD-group). Those with eGFR < 60 mL/min × 1.73 m² and UACR < 3.0 mg/mmol were assigned to the NA-CKD group. Patients with eGFR ≥ 60 mL/min × 1.73 m² and UACR ≥ 3.0 mg/mmol were defined as albuminuric with preserved renal function (A-CKD-group). Finally, the individuals with eGFR < 60 mL/min × 1.73 m² and UACR ≥ 3.0 mg/mmol comprised
the albuminuric CKD group (A-CKD+). The demographic and clinical characteristics of these groups are presented in Table 1.

Women made up a high proportion of NA-CKD patients ($P < 0.001$ compared with the CKD– group), while the proportion of men was highest in the A-CKD group ($P = 0.012$ compared with the CKD– group). Patients from the NA-CKD and A-CKD+ groups were older than CKD– and A-CKD– patients (all $P < 0.01$). Patients with NA-CKD demonstrated the lowest waist-to-hip ratio (WHR) among the examined T2D patients. In contrast, A-CKD– patients had the largest WHR values. There were no differences between the groups in body mass index (BMI). The percentage of current smokers was highest in the A-CKD+ group.

Patients with reduced renal function (NA-CKD and A-CKD+ groups) had longer diabetes durations than those without. The prevalence of diabetic retinopathy tended to be higher in A-CKD– and A-CKD+ patients, though the differences between the groups were not statistically significant. Despite the fact that the prevalence of coronary artery disease did not differ between the groups, myocardial infarction occurred more frequently in individuals with albuminuria (A-CKD– and A-CKD+ groups). In contrast, carotid atherosclerosis and a history of cerebrovascular events (stroke or transient ischemic attack) were most prevalent in patients with decreased eGFR (NA-CKD and A-CKD+ groups). The prevalence of peripheral artery disease was highest in A-CKD+ patients.

Most patients in our cohort were insulin-treated (Table 1). The duration of insulin therapy was longest in NA-CKD patients. Interestingly, the daily insulin dose in this group was lowest. All patients received antihypertensive agents, mostly in combinations. The highest frequency of the use of diuretics was observed among NA-CKD patients, while the highest rate of treatment with dihydropyridine calcium channel blockers (nifedipine or amlodipine) was found in the A-CKD+ group. Statins and antiplatelet agents were more commonly prescribed to NA-CKD and A-CKD+ patients.

Laboratory parameters of T2D patients depending on their CKD status are summarized in Table 2. The highest HbA1c and 2h-postprandial blood glucose levels were observed in the A-CKD+ group. The NA-CKD group was characterized by the lowest HbA1c values. Serum uric acid was increased significantly in CKD patients compared to those without, but no dependence on the CKD pattern was observed. As expected, the red blood cell (RBC) count was decreased in patients with reduced renal function (NA-CKD and A-CKD+ groups) compared to those without. The lowest hemoglobin levels were found in the NA-CKD group. The erythrocyte sedimentation rate (ESR) was increased significantly in all CKD groups compared to the CKD– group. No differences in other hematological parameters were revealed. The A-CKD+ patients demonstrated significantly increased levels of plasma SFMC compared to CKD– individuals. Fibrinogen and D-dimer levels did not differ between the groups.

Risk factors for CKD patterns
The risk factors for different CKD patterns are presented in Table 3. Age ≥ 65 years, duration of T2D ≥ 15 years, female sex, and the use of diuretics were significant risk factors for NA-CKD. On the other hand, male sex, smoking, WHR > 1.0 and HbA1c > 8.0% significantly increased the risk of A-CKD+. Diabetes duration ≥ 15 years and the use of dihydropyridine calcium channel blockers were associated with A-CKD+. In multiple logistic regression analysis, age, HbA1c, female sex and treatment with diuretics were significant predictors of decreased eGFR (Table 4). Meanwhile, WHR, HbA1c and male sex predicted elevated albuminuria (Table 5).

Urinary biomarkers in T2D patients with different patterns of CKD
The excretion of nephrin and podocin was increased significantly in all diabetic groups compared to control (all $P < 0.05$, Figure 2). The CKD– and NA-CKD groups did not differ in the levels of urinary excretion of nephrin or podocin. Patients with elevated albuminuria (A-CKD– and A-CKD+ groups) demonstrated an increase in the excretion of both markers compared to the CKD– group (A-CKD–: $P = 0.001$ and $P = 0.006$, respectively; A-CKD+: $P = 0.04$ and $P = 0.002$, respectively) and the NA-CKD group (A-CKD–: $P = 0.000003$ and $P = 0.0003$, respectively; A-CKD+: $P = 0.04$ and $P = 0.00007$, respectively).

The urinary excretion of WFDC-2 in men was 9.2 times higher than in women ($P < 0.000001$). Accordingly, sex differences in marker excretion were taken into account when evaluating the results (Figure 3). Men of the CKD–, NA-CKD and A-CKD+ groups demonstrated increased excretion of WFDC-2 compared to the nondiabetic control ($P = 0.04$, $P = 0.01$ and $P = 0.009$, respectively). However, there were no significant differences in this marker between diabetic groups. In women, WFDC-2 excretion was increased markedly in the NA-CKD and A-CKD+ groups compared to control ($P = 0.01$ and $P = 0.0007$, respectively) and to patients without CKD ($P = 0.01$).
Table 1  Clinical characteristics of type 2 diabetic individuals with different patterns of chronic kidney disease

| Parameter                              | CKD− (n = 89) | NA-CKD (n = 111) | A-CKD− (n = 87) | A-CKD+ (n = 73) |
|----------------------------------------|--------------|-----------------|----------------|----------------|
| General clinical parameters            |              |                 |                |                |
| Sex, M/F, n (%)                        | 20/69        | 13/98           | 45/42          | 22/51          |
| Age, yr                                | 64 (58.67)   | 68 (64.73)      | 63 (59.68)     | 67 (61.77)     |
| BMI, kg/m²                              | 33.4 (28.7; 36.9) | 32.6 (29.4; 37.2) | 33.6 (30.1; 38.2) | 33.4 (30.0; 36.8) |
| WHR                                    | 0.97 (0.94; 1.03) | 0.94 (0.89; 0.99) | 1.04 (0.97; 1.11) | 0.98 (0.95; 1.07) |
| Smoking, n (%)                         | 7 (7.9)      | 6 (5.4)         | 18 (20.9)      | 3 (4.1)        |
| Diabetes duration, yr                  | 15 (12; 19)  | 18 (15; 25)     | 15 (13; 20)    | 18 (14; 22)    |

Diabetic complications and comorbidities

| Parameter                              | CKD− (n = 89) | NA-CKD (n = 111) | A-CKD− (n = 87) | A-CKD+ (n = 73) |
|----------------------------------------|--------------|-----------------|----------------|----------------|
| Diabetic retinopathy, n (%)            | 62 (69.7)    | 74 (66.7)       | 65 (74.7)      | 57 (78.1)      |
| Arterial hypertension, n (%)           | 85 (95.5)    | 111 (100)       | 87 (98.9)      | 73 (100)       |
| Coronary artery disease, n (%)         | 41 (46.1)    | 58 (52.3)       | 46 (52.9)      | 41 (56.2)      |
| Myocardial infarction in anamnesis, n (%) | 7 (7.9) | 19 (17.1)       | 20 (23.0)      | 17 (23.3)      |
| Chronic heart failure (NYHA class III-IV), n (%) | 5 (5.6) | 7 (6.3)         | 11 (12.6)      | 4 (5.5)        |
| Carotid atherosclerosis, n (%)         | 15 (16.9)    | 51 (45.9)       | 33 (37.9)      | 40 (54.8)      |
| Cerebrovascular event in anamnesis, n (%) | 6 (6.7) | 13 (11.7)       | 5 (5.8)        | 11 (15.1)      |
| Peripheral artery disease, n (%)       | 60 (67.4)    | 84 (75.7)       | 59 (67.8)      | 57 (78.1)      |

Treatment

| Parameter                              | CKD− (n = 89) | NA-CKD (n = 111) | A-CKD− (n = 87) | A-CKD+ (n = 73) |
|----------------------------------------|--------------|-----------------|----------------|----------------|
| Metformin, n (%)                       | 61 (68.5)    | 64 (57.7)       | 56 (64.4)      | 43 (58.9)      |
| Sulfonylurea, n (%)                    | 29 (32.6)    | 31 (27.9)       | 21 (24.1)      | 10 (13.7)      |
| Insulin, n (%)                         | 74 (83.1)    | 94 (84.7)       | 76 (87.5)      | 70 (95.9)      |
| Duration of insulin therapy, yr        | 6 (4.10)     | 10 (7.13)       | 6 (3.10)       | 8 (5.11)       |
| Daily insulin dose, IU                 | 52 (56.72)   | 46 (34.62)      | 56 (40.78)     | 60 (42.74)     |
| Daily insulin dose, IU/kg              | 0.60 (0.40; 0.80) | 0.55 (0.40; 0.70) | 0.60 (0.40; 0.80) | 0.63 (0.45; 0.90) |
| RAS blockers, n (%)                    | 67 (75.3)    | 93 (83.8)       | 69 (79.3)      | 61 (83.6)      |
| Diuretics, n (%)                       | 36 (40.4)    | 73 (65.8)       | 38 (43.7)      | 35 (47.9)      |
| Calcium channel blockers, n (%)        | 27 (30.3)    | 38 (34.2)       | 34 (39.1)      | 36 (49.3)      |
| Antiplatelet agents, n (%)             | 46 (51.7)    | 78 (70.3)       | 50 (57.5)      | 57 (78.1)      |
| Statins, n (%)                         | 28 (31.5)    | 59 (53.2)       | 31 (35.6)      | 39 (53.4)      |

*p < 0.05, *p < 0.01, *p < 0.001 vs CKD−,
*bp < 0.001 vs A-CKD−,
*ap < 0.05, *bp < 0.01, *p < 0.001 vs NA-CKD,
*ap < 0.05, *bp < 0.001.

*Korbut AI. Risk factors and biomarkers of CKD in T2D. WJD 2019;10(11):523-532.

and P = 0.0007, respectively), while no difference between the CKD− group and the non diabetic control was found.

In diabetic patients, urinary excretion of nephrin and podocin correlated positively with UACR (r = 0.47, P = 0.000001 and r = 0.43, P = 0.00001, respectively). Nephrin excretion demonstrated positive relationships with age (r = 0.27, P = 0.0007), diabetes duration (r = 0.31, P = 0.0002), and SFMC (r = 0.37, P = 0.001); at the same time, podocin excretion was related to age only (r = 0.27, P = 0.0004). No relationships between podocyte-specific markers and eGFR were found (r = 0.47, P = 0.000001 and r = 0.43, P = 0.00001, respectively). In women with diabetes, the excretion of WFDC-2 correlated with serum creatinine levels (r = 0.45, P = 0.001), eGFR (r = 0.50, P = 0.001) and UACR (r = 0.45, P = 0.001). In males, WFDC-2 correlated with BMI and WHR (r = 0.52, P = 0.01 and r = 0.53, P = 0.04, respectively), but not with UACR (r = 0.18, P > 0.05) or eGFR (r = -0.13, P > 0.05).
Table 2  Laboratory parameters of type 2 diabetic individuals with different patterns of chronic kidney disease

| Parameter                        | CKD- (n = 89) | NA-CKD (n = 111) | A-CKD- (n = 87) | A-CKD+ (n = 73) |
|----------------------------------|---------------|------------------|----------------|----------------|
| **Renal tests**                  |               |                  |                |                |
| Serum creatinine, µmol/L         | 76 (67.3; 86.8) | 111 (99.1; 124) | 85.8 (76.1; 96.5) | 116 (97.8; 144) |
| eGFR, ml/min × 1.73 m²           | 77 (69; 87)   | 52 (46; 56)     | 72 (66; 84)   | 51 (46; 55)    |
| UACR, mg/mmol                   | 0.5 (0.3; 0.9) | 0.7 (0.4; 1.0)  | 8.3 (4.8; 36.7) | 11.4 (5.6; 42.1) |
| Urinary protein excretion, mg/day| 65 (50; 100)  | 70 (50; 140)    | 170 (90; 530) | 200 (130; 520) |
| **Biochemistry**                 |               |                  |                |                |
| HbA1c, %                         | 8.4 (7.5; 10.1) | 8.1 (7.2; 9.5)  | 9.7 (8.5; 11.2) | 8.6 (7.3; 9.8) |
| Hba1c, mmol/L                   | 68 (56; 87)   | 65 (55; 80)     | 83 (69; 99)   | 70 (58; 84)    |
| Fasting blood glucose, mmol/L    | 8.9 (6.8; 10.2) | 8.8 (6.5; 10.1) | 9.5 (8.0; 12.8) | 9.6 (7.7; 12.0) |
| 2h-postprandial blood glucose, mmol/L | 10.7 (9.0; 13.7) | 11.7 (8.9; 14.0) | 13.1 (9.9; 15.0) | 11.3 (10.0; 14.0) |
| Total cholesterol, mmol/L        | 5.1 (4.5; 5.9) | 5.1 (4.3; 6.0)  | 4.9 (4.1; 6.0) | 5.3 (4.1; 6.4) |
| LDL-cholesterol, mmol/L          | 3.3 (2.7; 3.9) | 3.2 (2.5; 4.0)  | 3.1 (2.5; 3.8) | 3.2 (2.5; 4.1) |
| HDL-cholesterol, mmol/L          | 1.2 (1.0; 1.4) | 1.3 (1.1; 1.5)  | 1.2 (1.0; 1.3) | 1.1 (1.1; 1.4) |
| Triglycerides, mmol/L            | 1.6 (1.3; 2.2) | 1.6 (1.1; 2.4)  | 1.8 (1.2; 2.9) | 1.8 (1.3; 2.8) |
| Uric acid, µmol/L                | 279 (218; 349) | 327 (269; 381)  | 324 (276; 376) | 349 (272; 390) |
| **Hematology**                   |               |                  |                |                |
| Hemoglobin, g/L                  | 137 (130; 144) | 129 (123; 140)  | 138 (126; 147) | 133 (123; 143) |
| RBC, × 10¹²/L                    | 4.8 (4.5; 5.0) | 4.5 (4.2; 4.8)  | 4.7 (4.5; 5.1) | 4.5 (4.1; 4.9) |
| WBC, × 10⁹/L                     | 6.5 (5.7; 8.0) | 6.7 (5.7; 7.8)  | 6.6 (5.3; 7.9) | 6.9 (5.7; 8.0) |
| Platelets, × 10⁹/L               | 238 (199; 270) | 234 (195; 270)  | 233 (191; 281) | 229 (189; 273) |
| ESR, mm/h                        | 16.5 (10; 23)  | 22 (15; 31)     | 22.5 (15.5; 29.5) | 23 (18; 33) |
| **Coagulation tests**            |               |                  |                |                |
| Fibrinogen, g/L                  | 4.4 (3.9; 5.5) | 4.4 (3.9; 5.1)  | 4.5 (3.8; 5.7) | 4.1 (3.7; 5.1) |
| SFMCs, mg/dL                     | 5.5 (3.5; 15)  | 12 (7; 16)      | 14 (8; 23)    | 12.5 (7; 21)   |
| D-dimer, mg/mL                   | 263 (235; 303) | 287 (239; 351)  | 271 (232; 304) | 290 (254; 363) |

*P < 0.05,
**P < 0.01,
###DISCUSSION

**Key findings**

The results of this study demonstrate the characteristics of different CKD course patterns in patients with long-term T2D. First, by matching a panel of clinical and laboratory parameters of T2D patients who had an increase in albuminuria or a decrease in eGFR, or both deviations, with those without, we identified the risk factors for these CKD patterns. Second, we showed some features in the urinary excretion of biomarkers, reflecting podocyte and interstitium involvement, in patients with T2D and different patterns of CKD. The data provide further evidence that albuminuric and NA-CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

**The risk factors for NA-CKD**

The development of NA-CKD in patients with T2D was associated with older age (≥ 65 years), female sex, longer diabetes duration (≥ 15 years), and the use of diuretics. Age over 65 years was a risk factor for both albuminuric and NA-CKD patterns in our
| Risk factor                  | NA-CKD (n = 111) | A-CKD– (n = 87) | A-CKD+ (n = 73) |
|-----------------------------|------------------|-----------------|-----------------|
| Age ≥ 65 yr                 | 3.16 (1.76-5.70) | 1.00 (0.55-1.80) | 1.76 (0.94-3.28) |
| Duration of diabetes ≥ 15 yr| 2.81 (1.53-5.17) | 1.63 (0.89-3.01) | 2.32 (1.19-4.53) |
| Male sex                    | 0.46 (0.21-0.98) | 2.32 (1.20-2.48) | 1.49 (0.74-3.01) |
| Female sex                  | 2.19 (1.02-4.69) | 0.43 (0.22-0.83) | 0.67 (0.33-1.36) |
| Smoking                     | 0.81 (0.25-2.60) | 3.49 (1.31-9.28) | 0.56 (0.13-2.34) |
| WHR >1.0                    | 0.61 (0.22-1.65) | 3.64 (1.32-9.99) | 1.53 (0.57-4.10) |
| HbA1c > 8.0%                | 0.68 (0.38-1.20) | 2.67 (1.35-5.27) | 1.10 (0.58-2.09) |
| Treatment with diuretics    | 2.80 (1.56-5.00) | 1.10 (0.60-2.00) | 1.30 (0.70-2.44) |
| Treatment with calcium channel blockers | 1.20 (0.66-2.17) | 1.47 (0.79-2.75) | 2.23 (1.17-4.25) |

The data are presented as odds ratio, 95% confidence interval and P value. CKD: Chronic kidney disease; CKD–: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD–: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; HbA1c: Hemoglobin A1c; WHR: Waist-to-hip ratio.

cohort. This finding may be explained by the general tendency for GFR to decrease in elderly patients\(^{(27)}\), as well as the inverse dependence of eGFR on age when calculated using the CKD-EPI formula\(^{(28)}\).

In our patients, female sex was a risk factor for NA-CKD, which is consistent with previous studies\(^{(29,30)}\). It should also be taken into consideration that in women, who predominated in the NA-CKD group, the CKD-EPI formula gives lower eGFR values than in men when operating with equal creatinine levels. It was recently revealed that in healthy individuals, single-nephron GFR demonstrated no differences between men and women, but the total GFR values in women are typically lower than those in men due to fewer nephrons in the female kidney\(^{(31)}\). The duration of diabetes for 15 years or more increased the risk of NA-CKD and A-CKD+ phenotypes, affecting eGFR more than albuminuria.

The proportion of patients taking diuretics was highest in the NA-CKD group. The relationships between diuretics and CKD require cautious interpretation. On the one hand, diuretics, especially in high doses, can cause deteriorative effects on the renal tubulointerstitium by provoking metabolic acidosis\(^{(32)}\), activation of the renin-angiotensin system\(^{(33)}\), or hypokalemia\(^{(34)}\). Recent data indicate that the use of diuretics is associated with adverse renal outcomes, indicated by a decline in eGFR and an increasing risk of renal replacement therapy initiation in CKD patients. It was speculated that reduced GFR can be the result of episodes of lowering blood pressure, volume depletion and related acute renal injury induced by diuretics, especially when used in combination with other antihypertensive agents\(^{(35)}\). The worsening of renal function in elderly patients with chronic heart failure has been associated with high doses of loop diuretics\(^{(36)}\). The more frequent use of diuretics in patients with reduced renal function may be attributed to more prevalent and/or advanced arterial hypertension, heart failure or other fluid retention syndromes, which occur before the start of diuretic therapy. In our cohort, no differences in the prevalence of arterial hypertension or congestive heart failure were observed between the groups. The role of diuretics as factors modifying the CKD course requires further research.
Risk factors for albuminuria not accompanied by eGFR reduction

In our study, male sex, smoking, WHR > 1.0 and HbA1c > 8.0% were identified as risk factors for albuminuria not accompanied by a decrease in eGFR. This CKD pattern was more common in men. In multivariate logistic regression analysis, male sex was a significant risk factor for albuminuria. These data are in agreement with results of other research indicating an increased risk of albuminuria in men with T2D[41-43].

The percentage of current smokers was highest in the A-CKD- group. The association between albuminuria and smoking has been reported previously[37,38,39]. It was demonstrated that smoking cessation contributes to the reduction of albuminuria in patients with newly diagnosed T2D[40]. The direct fibrogenic effect of tobacco smoke in the kidneys has been revealed in experimental CKD[41]. Other underlying mechanisms for the association between smoking and albuminuria have been proposed, including activation of the sympathetic and renin-angiotensin systems, an increase in blood pressure, changes in intraglomerular hemodynamics, progression of atherosclerotic changes, and activation of vascular-platelet interactions[39].

As expected, poor glycemic control turned out to be a risk factor for UACR elevation. The albuminuric effect of hyperglycemia has been linked with the accumulation of advanced glycation end-products, which, through the activation of protein kinase C and nuclear factor-κB, enhance the synthesis of fibrogenic and proinflammatory factors in glomerular and tubular cells[40]. These changes lead to deterioration of the glomerular endothelium and podocytes[41] and impair albumin reabsorption in the proximal tubules[42]. Recent studies have indicated that suppression of autophagy under hyperglycemic conditions promotes podocytopathy and increases the permeability of the glomerular filter[43].

The WHR was identified as another risk factor for albuminuria not accompanied by eGFR reduction. The relationship between abdominal obesity and albuminuria has been shown in a number of studies[37,38,39]. Hyperproduction of proinflammatory and fibrogenic cytokines, oxidative stress, and imbalances in adipokines are suggested as mechanisms of albuminuric effect in abdominal obesity[40]. The association between WHR and albuminuria could be mediated by insulin resistance. A study in db/db mice, a T2D model, showed that both albuminuria and glomerulosclerosis are related to insulin resistance[43]. A positive correlation has been found between homeostatic model assessment of insulin resistance index and the UACR values in patients with T2D[43]. Misregulation of epithelial proteins, such as nephrin and megalin, and activation of the mTOR/S6 kinase pathway seem to be involved in mediating the pathophysiology of insulin resistance, kidney hypertrophy, hyperfiltration and microalbuminuria[43].

Risk factors for albuminuric CKD

According to our results, diabetes duration and the use of calcium channel blockers were risk factors for albuminuric CKD. If the effect of the disease duration on the risk of CKD is quite natural, the relationships between dihydropyridines and CKD deserve discussion. On the one hand, initially more severe hypertension and the use of several antihypertensive drugs, there could be a combination of factors similar to the effects of diuretics, such as initially more severe arterial hypertension, and the use of several antihypertensive agents, which lead to a higher risk of arterial hypotension and prerenal acute kidney injury[38]. Another possible mechanism involves the dilatation of the vas afference and the intraglomerular hypertension that can be induced by nifedipine, or amlodipine, the most widely used L-type calcium channel blockers[45-47]. In our patient cohort, nifedipine and amlodipine were the only representatives of the class of calcium channel blockers. Meanwhile, observational studies have shown a reduction in albuminuria when patients were switched from L-
### Logistic regression model for urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol, logit(P) = \ln\left[\frac{P}{1-P}\right]

| Parameter     | Coefficient β | 95%CI      | P value |
|---------------|---------------|------------|---------|
| Constant      | -8.1206       | -13.1599, -3.0813 | 0.002   |
| WHR           | +5.1228       | 0.3920, 9.8535 | 0.03    |
| HbA1c, %      | +0.3570       | 0.1169, 0.5971 | 0.004   |
| Male sex, (1 or 0) | +0.6725     | 0.1920, 1.1531 | 0.006   |

Area under the receiver operating characteristic curve = 0.7612, P value for Kolmogorov-Smirnov statistics = 0.00004. CI: Confidence interval; HbA1c: Glycated hemoglobin; logit: Logit-function; WHR: Waist-to-hip ratio.

According to meta-analyses, antialbuminuric activity has been shown for nondihydropyridine, dihydropyridine L/N-, L/T- and T-type calcium channel blockers.

#### Urinary excretion of nephrin and podocin

In this study, we found that urinary excretion of nephrin and podocin was increased significantly in individuals with long-term T2D and correlated positively with UACR. These data are in agreement with the notion that podocytopathy is a key factor leading to the development of proteinuria and glomerulosclerosis in diabetic kidney disease[63]. Elevated excretion of nephrin and podocin, which are expressed in podocytes exclusively, may reflect more severe podocyte injury in albuminuric patients. It was demonstrated that the loss of the podocytes correlates with the levels of proteinuria in diabetic nephropathy[60]. Thus, increased urinary excretion of nephrin and podocin can be a sign of podocyturia, which is detected in diabetic kidney disease[64]. The correlation between these markers and the urinary concentrations of podocytes in diabetes has been shown previously[65]. In our study, the excretion of nephrin and podocin was increased dramatically in patients with elevated albuminuria, regardless of the concomitant decline in renal function, compared to patients without any signs of CKD or NA-CKD. This may indicate more advanced podocyte injury in T2D patients with albuminuric CKD.

#### Urinary excretion of WFDC-2

The serum levels of WFDC-2 (HE-4) were validated previously as a marker of tubulointerstitial fibrosis[25,26]. In this study, we investigated for the first time the urinary excretion of WFDC-2 in individuals with T2D. First, we found that excretion of WFDC-2 in men was markedly higher (approximately 10 times) than in women. These findings could be explained by the sexual differences in the expression of this molecule. Besides the kidneys, in males WFDC-2 is expressed in the epithelial cells of the epididymal and seminal ducts and the glandular epithelium of the prostate, i.e., in the organs that are related to the urinary tract anatomically. In women, WFDC-2 expression is detected in the fallopian tubes, endometrium, and Bartholin’s glands[24], which do not contact directly with the urinary system.

Excretion of WFDC-2 showed different relationships with CKD between men and women. In men with diabetes, excretion of WFDC-2 was increased in all groups, regardless of the presence or pattern of CKD. In women, WFDC-2 excretion was increased in the groups with declined eGFR only. The women had an inverse correlation between WFDC-2 and eGFR values and a direct correlation between WFDC-2 and UACR. At the same time, urinary excretion of WFDC-2 was not associated with excretion of nephrin or podocin. These data are in agreement with previous morphological studies indicating a close relationship between GFR and tubulointerstitial involvement rather than glomerulopathy[60,66].

#### Limitations

Our study is not without limitations. First, it is a cross-sectional study that does not prove causality. The natural intraindividual variability in eGFR and UACR values could be a source of some errors in classifying patients into groups. The recruitment of patients at one clinical center and the relatively small sample size could have led to a shift in the results of biomarker assessment with respect to the general diabetic population.

#### The remarks for clinical practice and future research

In this study, we demonstrate the differences in the clinical and laboratory response...
characteristics of albuminuric and NA-CKD in patients with long-term T2D. We found that female sex, older age, longer diabetes duration and diuretic use were associated with the NA-CKD phenotype. Meanwhile, male sex, smoking, abdominal obesity, and poor glycemic control were risk factors for albuminuria elevation not accompanied by a reduction in eGFR. It should be noted that some of the abovementioned risk factors are modifiable, especially those associated with albuminuria, which is important from clinical point of view. The predominant effect of antihyperglycemic drugs on albuminuria or GFR should be considered when choosing treatment for T2D patients with different CKD phenotypes.

To our knowledge, this is the first study addressing the diversity in urinary biomarkers in T2D patients with different CKD phenotypes. The different patterns of the shifts in the urinary excretion of the biomarkers of podocyte and tubulointerstitial involvement in albuminuric and NA-CKD give further support the notion that these phenotypes differ in their pathophysiology. A significantly more demonstrative increase in the excretion of nephrin and podocin in patients with elevated UACR compared to those without could suggest that albuminuric CKD is
Figure 3 Urinary excretion of WAP-four-disulfide core domain protein 2 in individuals with type 2 diabetes and different patterns of chronic kidney disease. A: Males; B: Females. *P < 0.05, **P < 0.01 vs non-diabetic control, ***P < 0.05, ****P < 0.01 vs CKD− group (the test of multiple comparisons of mean ranks). CKD−: The group of individuals with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; NA-CKD: Non-albuminuric chronic kidney disease, the group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio < 3.0 mg/mmol; A-CKD−: Group of patients with estimated glomerular filtration rate ≥ 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol; A-CKD+: Group of individuals with estimated glomerular filtration rate < 60 mL/min × 1.73 m² and urinary albumin-to-creatinine ratio ≥ 3.0 mg/mmol.

associated with more severe podocyte involvement. The increase in urinary excretion of WFDCC-2 in women with T2D and decreased eGFR apparently indicates more advanced tubulointerstitial lesion. The assessment of the predictive value of the studied biomarkers in different phenotypes of CKD is a challenge for future research. Studies of the renoprotective potential of antihyperglycemic, antihypertensive and other therapeutic agents in different CKD phenotypes are urgently needed. In conclusion, the data provide further evidence that albuminuric and NA-CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

ARTICLE HIGHLIGHTS

Research background
A number of researches show the heterogeneity of the natural course of chronic kidney disease (CKD) in patients with diabetes. Moreover, it has been shown that natural course of diabetic kidney disease is being transformed with increasing prevalence of declined renal function not accompanied by elevation of albuminuria. The trend is more evident in patients with type 2
diabetes (T2D). Currently, little is known about the mechanisms that determine the development of the albuminuric or nonalbuminuric phenotype of CKD. It was suggested that an increase in albuminuria may be a consequence of podocytopathy, while a decrease in renal function is associated with the involvement of tubulointerstitium.

**Research motivation**
The main topic of this study is in-depth clinical characteristics and identification of the risk factors and biomarkers of albuminuric and non-albuminuric CKD phenotypes in patients with T2D. The results may provide further progress in understanding of individual differences in the natural course of diabetic kidney disease and generation differentiated approaches to prevention and treatment of this complication.

**Research objectives**
The study aimed to identify the risk factors and urinary biomarkers of albuminuric and non-albuminuric CKD in patients with long-term T2D. Wherein, we tested the hypothesis that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression.

**Research methods**
Three hundred and sixty patients with T2D duration of at least 10 years from the date of diagnosis were included in this observational cross-sectional study. The associations of a panel of demographic and clinical characteristics, complications, comorbidities, and metabolic parameters with albuminuric and non-albuminuric CKD were analyzed. The urinary excretion of nephrin and podocin, two podocyte-specific markers, and WAP-four-disulfide core domain protein 2 (WFDC-2), a marker of tubulointerstitial fibrosis, was determined by ELISA in defined CKD phenotypes.

**Research results**
In this study we identified the risk factors of three CKD phenotypes in T2D patients. According to our data, non-albuminuric CKD is associated with age ≥ 65 years, female sex, diabetes duration ≥ 15 years, and the use of diuretics. Male sex, smoking, waist-to-hip ratio > 1.0 and HbA1c > 8.0% are risk factors for elevated albuminuria not accompanied by a decrease in estimated glomerular filtration rate (eGFR). Duration of diabetes ≥ 15 years and the use of calcium channel blockers seem to be risk factors for albuminuria with decreased eGFR. We also found some differences in predictors of decreased eGFR and increased albuminuria. In multivariate logistic regression analysis, age, HbA1c, female sex and diuretics were significant predictors for reduced eGFR, while waist-to-hip ratio, HbA1c and male sex were associated with elevated urinary albumin-to-creatinine ratio (UACR). In accordance with the tested hypothesis, we found the differences in urinary biomarkers of podocyte and tubulointerstitium involvement in patients with different CKD phenotypes. Excretion of nephrin and podocin was increased in patients with albuminuria, regardless of decline in renal function, correlating positively with UACR. At the same time, in women, WFDC-2 excretion was increased in those with reduced renal function, correlating negatively with eGFR.

**Research conclusions**
To our knowledge, this is the first study addressing the diversity in clinical characteristics and urinary biomarkers in T2D subjects with different CKD phenotypes. The results of this study provide new data on the risk factors and mechanisms of different variants of CKD in patients with long-term T2D. Firstly, by matching a panel of clinical and laboratory parameters of T2D patients who had an increase in albuminuria, a decrease in eGFR, or both deviations, with parameters in T2D patients with normoalbuminuria and preserved renal function, we showed the differences in profiles of the risk factors for CKD phenotypes. According to our data, non-albuminuric CKD phenotype is associated with age, female sex, diabetes duration, and the use of diuretics, whereas male sex, smoking, abdominal obesity and poor glycemic control are risk factors for elevated albuminuria. Secondly, we demonstrated some features in the urinary excretion of biomarkers, reflecting the podocyte and interstitial involvement, in patients with different CKD phenotypes. A significantly more demonstrative increase in the excretion of nephrin and podocin in patients with elevated UACR compared to those without could suggest that albuminuric CKD is associated with more severe podocyte involvement. The increase in urinary excretion of WFDC-2 in women with T2D and decreased eGFR apparently indicates more advanced tubulointerstitial fibrosis. The data provide further evidence that albuminuric and non-albuminuric CKD phenotypes correspond to different pathways of diabetic kidney disease progression. The diversity in the profiles of risk factors should be taken into account by clinicians in the management of diabetes.

**Research perspectives**
Since our study has a cross-sectional design, it does not prove causality. Accordingly, significance of some identified risk factors needs future confirmation. In particular, the role of abdominal obesity, insulin resistance, diuretics and calcium channel blockers needs to be verified in prospective studies. The assessment of the predictive value of the studied biomarkers in albuminuric and non-albuminuric CKD phenotypes is a challenge for future research. The studies of the renoprotective potential of antihyperglycemic, antihypertensive and other therapeutic agents in different CKD phenotypes are urgently needed.
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