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Associations of urinary, glomerular, and tubular markers with the development of diabetic kidney disease in type 2 diabetes patients

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Objectives: To evaluate the associations of urinary markers (eg albumin), glomerular (eg transferrin [TRF], immunoglobulin G [IgG]), and tubular (eg α1-microglobulin [α1-MG], β2-microglobulin [β2-MG]) markers with the development of diabetic kidney disease (DKD) in type 2 diabetes patients, as assessed by estimated glomerular filtration rate (eGFR) and albuminuria.

Material and methods: A total of 252 type 2 diabetes patients and 50 nondiabetic controls from Tianjin, China, were selected. Diabetic patients were divided into three groups according to eGFR levels, including groups A, B, and C with eGFR ≥90 (n=94), 60-89 (n=94), and 30-59 (n=64) mL/min/1.73 m^2. Urine levels of glomerular and tubular markers were detected in first morning urine samples, and their associations with eGFR and albuminuria analyzed.

Results: Urinary levels of IgG, TRF, and β2-MG were significantly elevated in diabetic patients with normal eGFR compared with nondiabetic control subjects. Urinary levels of all markers increased per eGFR stratum. All kidney damage markers were significantly associated with eGFR in univariate analysis (standard β between −0.35 and −0.28; all $P<.001$). After adjusting for known confounders, only the tubular markers α1-MG (standard β=−0.25; $P=.013$) and β2-MG (standard β=−0.18; $P=.039$) retained significant associations with eGFR. All kidney damage markers were significantly associated with albuminuria, independent of age, duration of diabetes, and eGFR (standard β between 0.45 and 0.86; all $P<.001$).

Conclusion: Only the tubular markers α_1-MG and β_2-MG were associated with eGFR independent of albuminuria, suggesting that they may play an important role in the development of DKD.

KEYWORDS
α1-microglobulin, β2-microglobulin, albuminuria, diabetic kidney disease, glomerular filtration rate

1 | INTRODUCTION

Diabetic kidney disease (DKD), the most common microvascular complication of diabetes mellitus (DM), is the leading cause of mortality and morbidity in DM patients.[1,2] According to current relevant guidelines, early screening and staging of DKD is based on both albuminuria and the glomerular filtration rate (GFR).[3] Albuminuria and GFR are generally considered non-invasive markers for DKD detection[4] and independent risk factors for cardiovascular events.[5] Classically, albuminuria is considered a consequence of diabetes-induced glomerular damage,[6] and the glomerulus has attracted attention from scientists as the major site of injury in DKD. However, recent studies emphasize

that tubulointerstitial damage might also play an important role in the pathophysiologic mechanisms implicated in DKD, especially in patients with type 2 diabetes.[7]

α1-microglobulin (α1-MG) and β2-microglobulin (β2-MG) are both low molecular weight proteins, and have been studied as markers of renal tubular dysfunction in various disorders.[8] Relatively few studies have assessed urinary α1-MG and β2-MG in diabetes, and these markers are not routinely used to diagnose and evaluate renal impairment in diabetic subjects.[9]

Relatively, albuminuria is of higher interest than GFR, because by definition, low GFR identifies only patients at a late disease stage.[10] However, recent findings emphasize the importance of early change of GFR and appropriate intervention; specifically, early intervention can significantly delay the development of diabetic kidney damage.[11,12] The Japan Diabetes Clinical Data Management study found that in normoalbuminuric type 2 diabetic patients, 20% have renal decline as assessed by GFR. Therefore, to explore the associations of glomerular and tubular damage markers with DKD, GFR may be even more valuable.

In this study, we assessed the urinary levels of tubular damage markers (α1-MG and β2-MG) and glomerular damage markers (transferrin and immunoglobulin G) in diabetic patients based on eGFR stratum as well as nondiabetic control subjects. The aim of this study was to evaluate the associations of these markers with kidney disease severity, as assessed by estimated glomerular filtration rate (eGFR), while exploring their relationships with albuminuria.

2 | MATERIALS AND METHODS

2.1 | Study population

A total of 252 type 2 diabetes patients were enrolled at the Department of Endocrinology and Nephrology, Tianjin Union Medical Center between March 2015 and January 2016. They were stratified by the eGFR level: group A, eGFR ≥90 mL/min/1.73 m^2 (n=94); group B, eGFR between 60 and 89 mL/min/1.73 m^2 (n=94); group C, eGFR between 30 and 59 mL/min/1.73 m^2 (n=64). Patients were excluded with cancer, urinary tract infections, a renal disease other than diabetic nephropathy, hemoglobin in urine, or pregnancy. As a control group, we included 50 nondiabetic subjects without renal disease and diabetes. Subjects were excluded from the control group with an eGFR <60 mL/min/1.73 m^2 or albumin-to- creatinine (ACR) >30 mg/g.

All procedures involving human participants were performed in accordance with the ethical standards of the Health bureau of Tianjin, and all participants provided written informed consent.

2.2 | Measurements

First morning urine and blood samples were obtained from subjects at clinical visits; meanwhile, medical history and anthropometric measurements were also recorded. Blood and urine samples were tested in our clinical laboratory department. All samples were stored at −80°C,

thawed at room temperature, and centrifuged before use. Serum cystatin C (CysC) and urinary proteins were assessed by nephelometry on a Dade-Behring BNII special protein analyzer, with manufacturer provided reagents. In this study, detection limits were: urinary albumin, 2.02 mg/L; α1-MG, 5.56 mg/L; β2-MG, 0.21 mg/L; transferrin (TRF), 2.17 mg/L; immunoglobulin G (IgG), 3.36 mg/L. These limits of detection were assigned to samples with concentrations lower than these values for each marker. For example, we assigned 5.56 mg/L to α_1-MG concentration lower than 5.56 mg/L. Urinary creatinine was measured by an enzymatic method on AbbottIC16000. We evaluated eGFR by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: for CysC ≤0.8 mg/L, eGFR=133 ×(CysC/0.8)$^{-0.499}$×0.996^{age} (female×0.932); for CysC>0.8 mg/L,eGFR=133×(CysC/0.8)$^{-1.328}$×0.996^{age}(female×0.932).[13] Meanwhile, ACR, α_1-microglobulin-to-creatinine ratio (α_1-MG/Cr), β2-microglobulin-to-creatinie ratio (β2-MG/Cr), transferrin-to-creatinine ratio (TRF/Cr), and immunoglobulin G-microglobulin-to-creatinine ratio (IgG/Cr) were calculated.

2.3 | Statistical analysis

Analyses were performed with SPSS 20.0 (IBM Corporation, Armonk, NY, USA). Data with skewed distribution were expressed as median and inter-quartile range, and normally distributed data as mean±SD. Differences among groups were tested by ANOVA for normally distributed variables and Chi-square test for categorical variables. For non-normally distributed variables, Kruskal–Wallis test was used. Logarithmic transformation of non-normally distributed variables was applied. Diabetic patients were divided into two groups, based on median cut-off values of urinary markers. Multivariate logistic regression analysis was used to evaluate the impact of clinical parameters on elevated urinary markers. We performed a linear regression analysis by using eGFR and ACR as dependent variables, respectively, and various damage markers as independent variables, to investigate the urinary markers related to eGFR and albuminuria. $P<.05$ was considered statistically significant.

3 | RESULTS

Table 1 shows the baseline characteristics of all subjects. Compared with nondiabetic subjects, group B and C patients were slightly older and had lower level of eGFR; group C had higher prevalence of hypertension and increased level of diastolic blood pressure (DBP). Patients in groups C and B more often had a history of cardiovascular disease and lower levels of DBP and eGFR amounts, compared with group A individuals. Meanwhile, HbA1c levels were higher in groups A and C than in group B.

The levels of damage markers in nondiabetic control subjects and diabetic patients are shown in Table 2. β2-MG, IgG, and TRF amounts were higher in patients with diabetes of group A compared with values of nondiabetic control subjects. ACR and α1-MG levels were higher in patients with diabetes of group B than in control subjects; however,

TABLE 1 Characteristics of nondiabetic control subjects (n=50) and diabetes patients (n=252) according to eGFR stratum

		Subjects with diabetes			P value in diabetes
	Nondiabetic subjects	A	B	C	
Number	50	94	94	64	—
Age (y)	58.9±2.9	59.3±10.6	64.9±10.8[*]	67.6±10.7[*]	<.001
Male gender (%)	47.4	58.5	63.8	56.3	.596
Diabetes duration (y)	—	6 (2-10)	8 (2-16)	8 (3-15)	.109
History of cardiovascular disease (%)	0	36.2	55.3	63.5	.002
Hypertension (%)	47.4	63.0	65.9	73.4[*]	.389
BMI (kg/m^2)	24.5±3.0	26.3±4.5	25.4±3.5	24.9±5.9	.298
Systolic blood pressure (mm Hg)	133.9±25.7	134.1±11.6	135.5±14.5	132.5±11.5	.351
Diastolic blood pressure (mm Hg)	81.9±11.6	80.9±8.3	79.2±7.9	76.7±7.6[*]	.006
HbA1c (%)	—	8.6±2.2	7.7±1.7	8.0±1.6	.032
eGFR (mL/min/1.73 m^2)	110.9±11.6	105.6±13.8	73.9±9.1[*]	47.8±7.9[*]	<.001

[*]P<.05 vs nondiabetic control subjects, calculated using the independent-samples t test for normal distributed variables and the Mann–Whitney U test for nonnormal distributed variables. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or use of antihypertensive medication.

TABLE 2 The level of damage markers in nondiabetic control subjects and in diabetes patients according to eGFR stratum

		Subjects with diabetes			P value in diabetes
	Nondiabetic subjects	A	B	C	
ACR (mg/g)	15.4 (12.8-24.9)	22.7 (13.8-41.2)	45.7 (16.1-143.3)[*]	71.4 (23.6-442.3)[*]	<.001
Glomerular					
IgG/Cr (mg/g)	5.5 (3.2-7.4)	6.9 (4.6-10.8)[*]	10.1 (5.2-17.8)[*]	18.9 (8.5-64.6)[*]	<.001
TRF/Cr (mg/g)	2.8 (1.9-4.3)	3.5 (2.4-6.2)[*]	5.2 (2.6-8.8)[*]	8.2 (3.7-22.4)[*]	<.001
Tubular					
α_1-MG/Cr (mg/g)	11.9 (8.6-20.7)	16.3 (10.3-29.4)	20.0 (11.2-41.0)[*]	36.0 (18.2-67.8)[*]	<.001
β_2-MG/Cr (mg/g)	0.3 (0.2-0.5)	0.5 (0.3-0.9)[*]	0.5 (0.3-1.8)[*]	1.1 (0.4-5.8)[*]	<.001

[*]P<.05 vs nondiabetic control subjects, calculated using the independent-samples t test for normal distributed variables and the Mann–Whitney U test for nonnormal distributed variables.

there were no statistically significant differences between group A and the control group. Meanwhile, the levels of all urinary damage markers increased with increasing amounts of eGFR in patients with diabetes.

The eGFR was negatively correlated with urinary IgG (r=−.35, P<.001), TRF (r=−.28, P<.001), α1-MG (r=−.32, P<.001), β2-MG (r=−.30, P<.001), ACR (r=−.33, P<.001) (Table 3 and Figure 1). The eGFR was correlated with age, DBP, BMI, duration of diabetes, HbA1c, and ACR. When these clinical parameters were entered in multivariate regression analyses, the tubular markers α_1-MG (Standard β=−0.25, P=.013) and β_2-MG (Standard β=−0.18, P=.039) remained correlated with eGFR independently of albuminuria, whereas all glomerular damage markers lost significance (Table 3).

Albumin-to-creatinine was positively correlated with urinary IgG (r=.86, P<.001), TRF (r=.83, P<.001), α1-MG (r=.61, P<.001), and β2-MG (r=.51, P<.001; Table 4 and Figure 2). All the damage markers were positively correlated with albuminuria in univariate regression analysis (Table 4). Meanwhile, albuminuria was correlated with age, duration

TABLE 3 Regression analyses of eGFR as a dependent variable in type 2 diabetes patients

	Univariate		Multivariate[b]	
Urinary markers	Standard β	P	Standard β	P
IgG/Cr[a]	−0.35	<.001	−0.29	.059
TRF/Cr[a]	−0.28	<.001	−0.10	.474
α_1-MG/Cr[a]	−0.32	<.001	−0.25	.013
β_2-MG/Cr[a]	−0.30	<.001	−0.18	.039
ACR[a]	−0.33	<.001	—	—

[a]Logarithm-transformed values were used for analysis.
[b]Adjustment for age, DBP, BMI, duration of diabetes, HbA1c, and ACR.

of diabetes, eGFR. When these clinical parameters were entered into multivariate regression analyses, the urinary markers were still associated with albuminuria (Table 4).
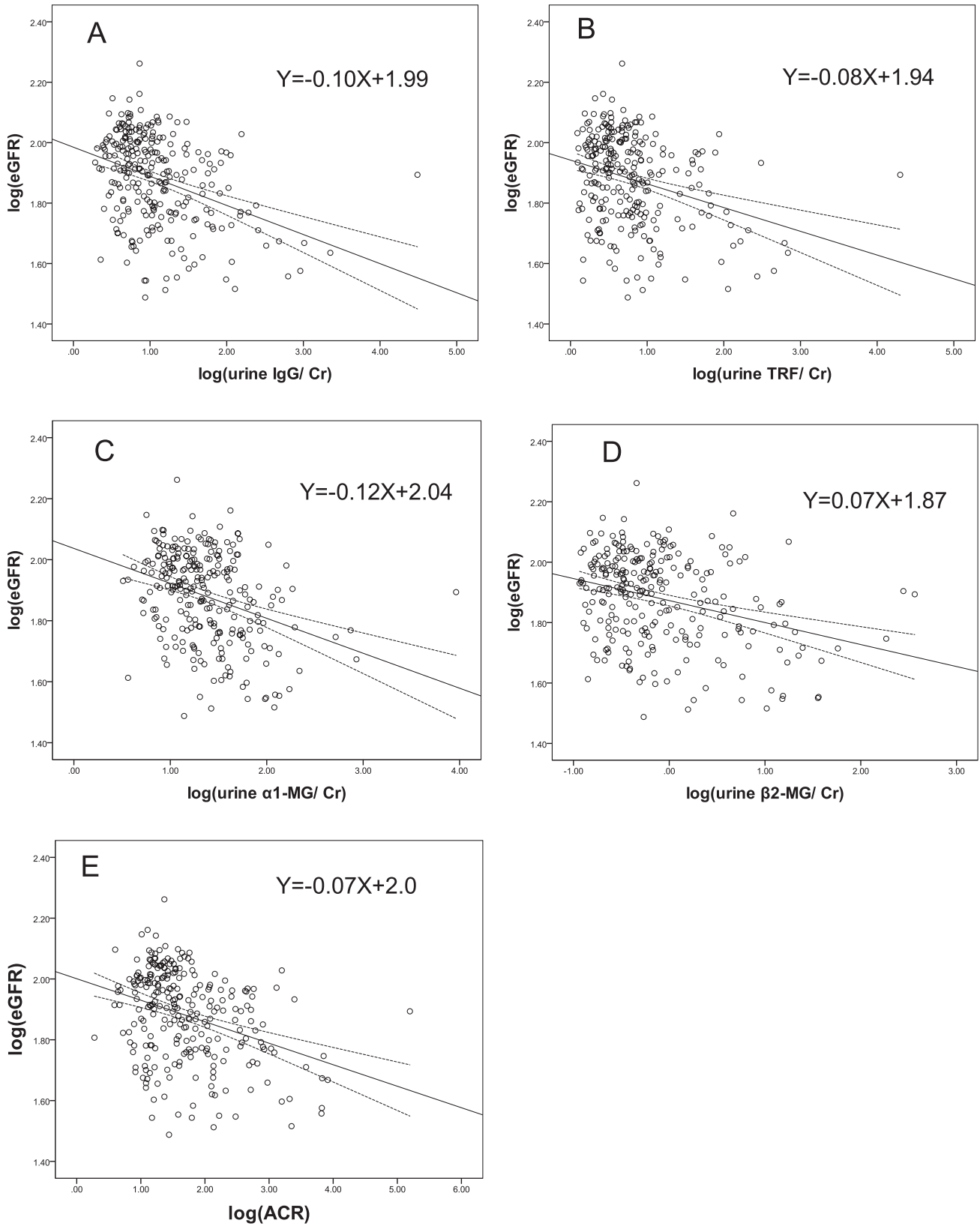
FIGURE 1 Single regression analyses of urinary estimated glomerular filtration rate in relation to urinary IgG, transferrin (A and B) and α1-MG, β2-MG (C and D), and albumin-to- creatinine (E) respectively. Logarithm-transformed values were used for analysis. Regression lines are represented by a 95% confidence interval
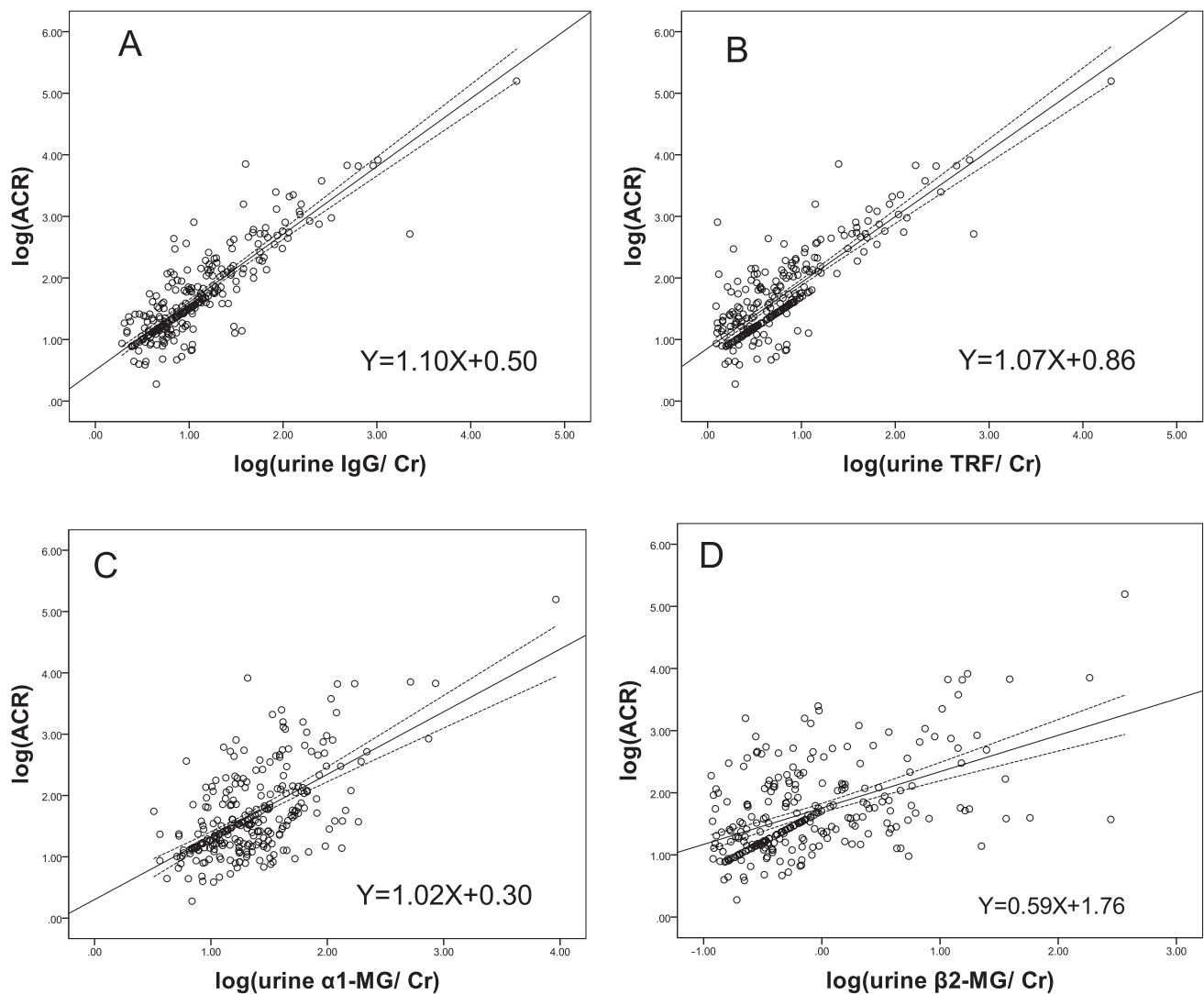
TABLE 4 Regression analyses of urinary ACR as a dependent variable in type 2 diabetes patients

Urinary markers	Univariate		Multivariate[b]	
	Standard β	P	Standard β	P
IgG/Cr[a]	0.86	<.001	0.86	<.001
TRF/Cr[a]	0.83	<.001	0.81	<.001
α_1-MG/Cr[a]	0.61	<.001	0.56	<.001
β_2-MG/Cr[a]	0.51	<.001	0.45	<.001

[a]Logarithm-transformed values were used for analysis.
[b]Adjustment for Age, duration of diabetes, eGFR.

diagnosis and intervention to prevent or delay kidney damage and reduce end-stage renal disease as well as cardiovascular events has an important clinical significance.[14] In the current study, we assessed glomerular and tubular damage markers to explore their associations with the development of diabetic kidney disease as assessed by eGFR. We found that urinary levels of glomerular and tubular damage markers were higher in patients with slightly decreased eGFR (30 mL/min/1.73 m^2≤eGFR≤90 mL/min/1.73 m^2). Interestingly, even in diabetic patients with relatively normal renal function (eGFR ≥90 mL/min/1.73 m^2), some markers like IgG, TRF, and β_2-MG were already elevated compared with the values obtained for nondiabetic control

FIGURE 2 Single regression analyses of urinary albumin-to-creatinine in relation to urinary IgG, transferrin (A and B) and α_1-MG, β_2-MG (C and D) respectively. Logarithm-transformed values were used for analysis. Regression lines are represented by a 95% confidence interval

4 | DISCUSSION

Early decline of GFR is closely related to the incidence and development of kidney disease and cardiovascular events; therefore, early

subjects. In regression analyses, all damage markers were significantly negatively associated with eGFR and significantly positively associated with albuminuria. Unlike glomerular damage markers, the tubular damage markers α_1-MG and β_2-MG remained negatively significantly

associated with eGFR after adjustment for albuminuria and other confounding factors.

In this study, urinary IgG and TFR levels were increased over the three eGFR groups, and negatively associated with eGFR in diabetic patients. The associations of glomerular damage markers (IgG, TFR) with DKD have been previously described, but most researches showed that the urinary levels of IgG and TFR paralleled the degree of urinary albumin excretion.[15–17] Mohan et al.[18] suggested that IgG levels represent the seriousness of kidney lesions. However, as shown above, IgG levels were increased in patients with relatively normal eGFR level compared with the values of nondiabetic control subjects, suggesting that IgG may appear in the early stage of DKD assessed by eGFR.

In general, all investigated tubular markers were increased in higher eGFR strata. Few studies have, to date, investigated the associations of the tubular markers α_1-MG and β_2-MG with the severity of kidney injury in DKD assessed by eGFR. Urinary α_1-MG was significantly increased in diabetic patients compared with normal subjects.[19] Urinary α_1-MG plays an important role in predicting the progression of DN as suggested by increased albuminuria.[9, 20] Increased excretion of β_2-MG was found in early disease course, while albumin excretion remained in the normal range in urine samples from diabetic patients.[20–22] In the current study, β_2-MG levels were elevated in diabetic patients with relatively normal renal function (eGFR ≥90 mL/min/1.73 m^2) compared with nondiabetic control subjects. These data suggested a role for the tubulointerstitium in the pathogenesis and early development of renal damage in patients with diabetes.

All markers were negatively associated with eGFR and positively correlated with albuminuria in univariate regression analyses. After adjustment for age, duration of diabetes, eGFR, all investigated markers remained correlated with albuminuria. However, the associations of eGFR with urinary IgG and TRF levels lost significance after adjusting for age, DBP, BMI, duration of diabetes, HbA1c, and albuminuria. In contrast, the levels of the tubular damage markers α_1-MG and β_2-MG remained associated with eGFR after adjusting for the above confounding factors. These data suggested that urinary α_1-MG and β_2-MG levels are promising markers for predicting the risk of DKD in addition to albuminuria.

In conclusion, the urinary levels of all the investigated damage markers were elevated in patients with diabetes compared with nondiabetic control subjects. These damage markers were associated with the severity of diabetic nephropathy as assessed by eGFR. Interestingly, some markers were already elevated in normal eGFR diabetic patients. This suggested these proteins as potential sensitive markers for early diabetic kidney damage. Only the tubular markers α_1-MG and β_2-MG were negatively associated with eGFR independently of albuminuria, suggesting that α_1-MG and β_2-MG may play an important role in the development of DKD. Furthermore, measuring urinary α_1-MG and β_2-MG concentrations may be useful in assessing the severity of diabetic kidney damage in addition to measuring albuminuria. However, further longitudinal studies are required to fully assess the potential clinical use of these promising biomarkers in a larger population.

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