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Original Article

Increased serum galectin-3 levels are associated with the development of type 2 diabetic nephropathy: a novel marker for progression?

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Abstract: Objective: Diabetic nephropathy (DN) is the most common cause of end-stage renal disease. The aim of this study was to evaluate the relationship between serum galectin-3 concentration and renal impairment in patients with type 2 DN. Methods: In this cross-sectional study, 30 patients with type 2 diabetes mellitus (DM), 120 patients with type 2 DN and 30 healthy control subjects were enrolled from August 2015 to April 2016. Serum galectin-3 levels were measured by enzyme-linked immunosorbent assay. The relationship between galectin-3 concentration and clinical variables was evaluated by multiple linear regression models. Results: Compared to healthy controls, serum galectin-3 levels were higher in patients with type 2 DM and DN. Galectin-3 showed a significant association with serum creatinine, β2-microglobulin, fasting plasma glucose, urine albumin creatinine ratio (UACR), age, hemoglobinA1c, systolic blood pressure and estimated glomerular filtration rate (eGFR). In a stepwise regression analysis, serum galectin-3 levels were negatively correlated with eGFR (β=-0.914, P<0.001), and positively correlated with UACR (β=0.394, P=0.011). Conclusions: Increased serum galectin-3 levels were independently associated with decreased eGFR and increased UACR in patients with type 2 DN.

Keywords: Diabetes, diabetic nephropathy, galectin-3

Introduction

Diabetic nephropathy (DN) is one of the major complications and the leading cause of end-stage renal disease (ESRD) in patients with type 2 diabetes mellitus (DM) [1, 2]. The pathogenesis of DN is multifactorial and a topic of intense research. Despite aggressive and comprehensive treatment, many patients with DN still develop ESRD. Reasons for this poor clinical outcome include lack of specific curative treatments and inadequate biomarkers that identify early more treatable stages of DN. Albuminuria and estimated glomerular filtration rate (eGFR) are currently the best biomarkers for the prediction of DN progression, although they have their limitations. Cross-sectional studies have shown that some DN patients with reduced renal function or even advanced renal disease do not present with significant albuminuria [3]. Calculation of eGFR is limited by variations in serum creatinine levels caused by differences in muscle mass, meat intake, age, gender, and renal pathological conditions. These limitations have stimulated the search to identify more reliable biomarkers that predict the progression of DN.

Galectin-3 is a 32- to 35-kDa water soluble β-galactosidase-binding glycoprotein that is a member of the multifunctional lectin family. It contains a C-terminal end with a carbohydrate recognition domain and an N-terminal domain [4]. Galectin-3 is mainly expressed by activated macrophages, mast cells and eosinophils, and less so by other tissues such as the epithelium of the gastrointestinal and respiratory tracts. It is expressed in the cytoplasm, nucleus, and cell surface, and is secreted into the extracellular spaces [5]. Galectin-3 plays important regulatory roles in a variety of pathophysiological processes, including fibrogenesis, tissue repair, and inflammation [6]. It may be involved in the pathogenesis of several diseases, such as can-

cer, diabetes, heart failure, as well as renal disease [7-10].

Galectin-3 has been shown to play a role in the initiation and development of non-diabetic nephropathy [11]. Recent studies confirmed that galectin-3 promotes the development of tubulointerstitial fibrosis in mouse models and humans [12, 13]. Galectin-3 expression has a close relationship with kidney function in patients with chronic kidney disease (CKD). It has been observed that elevated serum galectin-3 levels precede the development of CKD in the general population [14], and that circulating galectin-3 concentrations increase in parallel with decreasing kidney function [7]. However, the pathogenesis of DN is significantly different from that of other CKDs and whether the same association occurs in type 2 DN is not clear. While several reports have documented the strong relationship between increased serum galectin-3 levels and loss of kidney function [14-18], few have investigated changes in galectin-3 expression found in patients with DN [19], and none have analyzed the relationship between galectin-3 levels and different stages of DN.

The purpose of this cross-sectional study was to examine the clinical relevance of serum galectin-3 levels at various stages of type 2 DN and evaluate the association between galectin-3 levels and loss of renal function.

Materials and methods

Subjects

Thirty patients with type 2 DM and 120 patients with type 2 DN were enrolled between August 2015 and April 2016 from the Fourth Clinical Medical College of Guangzhou University of Chinese Medicine. Inclusion criteria were male or female older than 18 years of age, and medical diagnosis of type 2 DM or type 2 DN. Patients with infectious disease, inflammatory disease, liver disease, malignancy, heart failure, and patients undergoing renal replacement therapy (dialysis or kidney transplantation) were excluded from these groups. Thirty healthy, age-matched subjects without any medical disease or medical drug treatment were recruited as controls. All patients provided written informed consent prior to being enrolled in the trial. Study procedures were approved by the Ethics Committee of Shenzhen Affiliated Hospital of Guangzhou University of Chinese Medicine.

Clinical data collection

Type 2 DM was diagnosed according to the guidelines of the American Diabetes Association in 2013 [20]. The diagnosis and classification of DN CKD stages were established according to the criteria of the National Kidney Foundation K/DOQI Clinical Practice Guidelines for Chronic Kidney Disease [21]. The stages of DN were categorized using Mogensen DN diagnostic criteria [22]. Stage 1-2 DN was defined as hyperfiltration, with eGFR exceeding the upper limit of the reference range (120 ml/min per 1.73 m^2). These patients had normal albumin urinary excretion and no signs of clinical renal disease. Stage 3 DN was characterized by abnormally elevated urinary albumin excretion, also known as the “microalbuminuria”. Microalbuminuria was defined as urinary albumin excretion between 30-299 mg/day (or between 30-299 mg/g creatinine) on a random urine sample. Stage 4 DN was characterized as overt DN, also known as the “macroalbuminuria”. Macroalbuminuria was defined as urinary albumin excretion equal or greater than 300 mg/day (or above 300 mg/g creatinine) on a random urine sample. Stage 5 DN was known as ESRD. ESRD was defined as eGFR less than 15 ml/min per 1.73 m^2.

Patient clinical parameters were obtained, including gender, age, duration of DM, medications administered, body weight, height, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP). BMI was calculated as weight in kilograms divided by height in meters squared. SBP and DBP were reported as the average of three measurements taken in the sitting position with a standard mercury sphygmomanometer after a 5-minute rest. Blood test included determination of serum galectin-3, serum creatinine (Scr), fasting plasma glucose (FPG), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and β2-microglobulin (β2-MG) concentrations. Blood hemoglobinA1c (HbA1c), and urine albumin creatinine ratio (UACR) were also determined. Testing was performed using conventional laboratorial tech-

Table 1. Baseline demographic and clinical characteristics

Variables	Health Control groups	DM	DN (1-2)	DN3	DN4	DN5	P Value*	Total of DM and DN Patients
Number	30	30	30	30	30	30		150
Gender (male) (n,%)	14, 46.7%	16, 53.3%	16, 53.3%	20, 66.7%	19, 63.3%	16, 53.3%	0.721	87, 58%
Age (years)	52.8±7.1	53.2±11.4	52.2±13.0	54.1±9.9	60.1±13.5	63.7±9.6	p<0.001	56.7±12.3
Weight (kg)	60.1±9.2	68.7±12.6	63.2±9.3	67.9±12.5	65.7±10.8	63.2±11.4	0.191	65.7±11.5
Duration of diabetes (years)	-	8.5 (4.6-12.3)	6.0 (3.8-10.3)	8.0 (5.8-14.3)	10.0 (8.8-17.0)	12.0 (7.0-19.0)	0.007	10 (6-15)
FPG (mg/dL)	94.2 (88-98.9)	134.6 (112.5-163.4)	161.1 (116.1-233.1)	143.3 (112.3-190.3)	108.7 (87.9-162.5)	87.9 (68.5-116.4)	p<0.001	123.7 (98.1-175.1)
HbA1c (%)	-	7.8 (6.7-10.0)	9.7 (7.3-12.9)	7.3 (6.7-9.4)	8.7 (7.1-10.1)	6.2 (5.7-6.7)	p<0.001	7.6 (6.5-9.8)
TC (mg/dL)	173.9±24.7	157.7±32.4	171.9±43.5	175.7±42.8	204.1±64.6	179.3±56.5	0.09	177.7±50.9
TG (mg/dL)	92.5 (71.2-117.9)	126.7 (88.3-176.0)	151.4 (98.3-177.6)	112.5 (89.7-182.0)	134.6 (99.2-232.9)	152.8 (112.3-228.5)	0.297	132.0 (96.5-182.5)
LDL-C (mg/dL)	115.2±24.2	100.2±29.5	113.5±34.2	111.8±34.9	129.8±58.4	107.3±45.9	0.092	112.5±42.5
HDL-C (mg/dL)	55.8±9.9	43.8±11.2	38.4±11.3	47.2±14.5	46.7±14.6	39.4±17.3	0.042	43.1±14.2
Scr (mg/dL)	0.71 (0.67-0.85)	0.78 (0.61-0.87)	0.61 (0.52-0.65)	0.81 (0.67-0.89)	1.41 (1.05-1.84)	5.28 (4.45-7.02)	p<0.001	0.85 (0.64-1.84)
eGFR (ml/min/1.73 m^2)	97.8 (93.3-103.4)	107.4 (97.5-113.3)	141.0 (137.9-160.5)	104.4 (96.2-117.0)	49.0 (38.0-73.8)	11.0 (7.7-14.5)	p<0.001	96.6 (38.0-119.0)
β2-MG (mg/L)	-	0.11 (0.05-0.15)	0.18 (0.09-0.31)	0.18 (0.08-0.45)	1.57 (0.32-5.52)	26.26 (12.76-46.13	p<0.001	0.29 (0.10-4.18)
UACR (mg/g)	-	2.9 (1.3-4.0)	4.0 (0.4-10.8)	50.4 (32.5-72.1)	1311.6 (975.1-3149.6)	2918.6 (2393.3-4652.0)	p<0.001	9.5 (2.8-241.2)
Galectin-3 (ng/mL)	2.10 (1.50-2.68)	2.40 (1.69-3.39)	2.16 (1.91-2.97)	2.33 (1.74-3.48)	2.54 (2.04-3.53)	5.92 (4.66-7.35)	p<0.001	2.70 (2.01-4.49)
SBP (mmHg)	117±13	126±19	118±13	130±22	150±31	155±25	p<0.001	136±26
DBP (mmHg)	76 (70-83)	74 (68-84)	75 (67-77)	77 (70-90)	81 (74-90)	81 (73-91)	0.028	77 (71-86)
BMI (kg/m^2)	22.5 (21.0-24.3)	25.1 (22.8-26.5)	23.2 (22.0-24.6)	24.4 (21.8-27.1)	25.2 (22.8-27.1)	24.1 (19.8-26.3)	0.183	24.2 (22.1-26.3)
Hypertension (n, %)	0, 0%	11, 36.7%	13, 43.3%	15, 50.0%	13, 43.3%	16, 53.3%	0.728	68, 45.3%
CAD (n, %)	0, 0%	3, 10.0%	2, 6.7%	2, 6.7%	3, 10.0%	4, 13.3%	0.894	14, 9.3%
ACEI or ARB (n, %)	0, 0%	8, 26.7%	11, 36.7%	16, 53.3%	18, 60.0%	12, 40.0%	0.069	65, 43.3%
Insulin (n, %)	0, 0%	18, 60.0%	18, 60.0%	18, 60.0%	20, 66.7%	18, 60.0%	0.978	92, 61.3%
DPP-4 inhibitor (n, %)	0, 0%	12, 40.0%	10, 33.3%	9, 30.0%	11, 36.7%	10, 33.3%	0.943	52, 34.7%
Sulfonylurea (n, %)	0, 0%	10, 33.3%	11, 36.7%	13, 43.3%	10, 33.3%	9, 30.0%	0.854	53, 35.3%
Metformin (n, %)	0, 0%	15, 50.0%	12, 40.0%	13, 43.3%	8, 26.7%	7, 23.3%	0.057	55, 36.7%
Statin (n, %)	0, 0%	13, 43.3%	15, 50.0%	19, 63.3%	19, 63.3%	18, 60.0%	0.42	84, 56.0%

*Kruskal Wallis H test between the total 150 patients. Data are presented as frequencies and percentages for categorical variables, mean ± SD for normally distributed continuous variables, or median (25th; 75th percentiles) for non-normally distributed continuous variables. Based on Bonferroni's correction, a P-value <0.01 was considered statistically significant. Abbreviatons: FPG, fasting plasma glucose; HbA1c, hemoglobinA1c; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein. cholesterol; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; β2-MG, β2-microglobulin; UACR, urine albumin creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; CAD, coronary artery disease; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin II receptor blockers; DPP-4, dipeptidyl peptidase 4.

Table 2. Spearman's correlation between serum galectin-3 levels and clinical parameters in all DN patients (n=120, without DM)

Variable	Galectin-3	
	R	P Value
Age (years)	0.384	p<0.001
Gender	0.080	0.388
Duration of diabetes (years)	0.051	0.578
FPG (mg/dL)	-0.412	p<0.001
HbA1c (%)	-0.291	0.001
TC (mg/dL)	-0.044	0.630
TG (mg/dL)	0.024	0.799
LDL-C (mg/dL)	-0.061	0.507
HDL-C (mg/dL)	-0.134	0.144
Scr (mg/dL)	0.581	p<0.001
eGFR (ml/min/1.73 m^2)	-0.560	p<0.001
β2-MG (mg/L)	0.580	p<0.001
UACR (mg/g)	0.324	0.005
SBP (mmHg)	0.419	p<0.001
DBP (mmHg)	0.135	0.142
BMI (kg/m^2)	-0.070	0.452

Abbreviatons: FPG, fasting plasma glucose; HbA1c, hemoglobinA1c; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; β2-MG, β2-microglobulin; UACR, urine albumin creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

niques. The eGFR was calculated using the modification of diet in renal disease (MDRD) equation: eGFR (ml/min per 1.73 m^2) = $186*SCr^{-1.154}*Age^{-0.203}*0.742$ (if female) [23].

Laboratory methods

All blood samples were obtained in the morning after an overnight fast. Within 1 hour all blood samples were centrifuged at 1000 g at 4°C for 15 minutes and the serum immediately transferred into 1.5 ml cryotubes. All samples were stored at -80°C until they were tested. An enzyme-linked immunosorbent assay (ELISA) was used to determine serum galectin-3 levels, according to the manufacturer's instructions (Catalog No: SK00199-01, Aviscera Bioscience, Santa Clara, CA, USA). This assay has a high sensitivity to serum galectin-3 with a minimum detectable concentration of 10 pg/ml.

Statistical methods

Demographic characteristics were assessed using standard descriptive statistics. Continuous data was expressed as either the mean ± standard deviation or the median (interquartile range), depending on their distribution. Categorical data were expressed as numbers and percentages. Normally distributed continuous variables were compared using one-way ANOVA. Non-normally distributed continuous variables were compared using the Kruskal-Wallis test. The Chi-square test was used to compare categorical variables. Spearman's correlation coefficient was used to analyze associations between quantitative variables. Clinical variables that correlated with serum galectin-3 levels in DN patients were evaluated using univariate linear regression analysis. Variables that were significantly associated with galectin-3 levels in univariate linear regression model were further tested for independency in multivariate forward stepwise regression analysis. Skewed variables were log transformed before statistical analysis. All statistical procedures were performed using the SPSS statistical software package, version 17.0 (Chicago, IL, USA). A P value less than 0.05 was considered statistically significant. Bonferroni's correction for statistical significance of association was applied to compensate for multiple comparisons.

Results

Demographic characteristics of study subjects

Physical and clinical characteristics of subjects with type 2 DM (n=30), type 2 DN (n=120) and healthy control individuals (n=30) are shown in **Table 1**. Among 30 DM patients and 120 DN patients, there were no overt differences in gender (P=0.721), weight (P=0.191), BMI (P=0.183), TC (P=0.09), TG (P=0.297), and LDL-C (P=0.092) levels. Prevalence rates of cardiovascular and cerebrovascular disease were not different [hypertension (P=0.728), coronary artery disease (P=0.894)]. The frequency of drugs prescribed to treat the diabetes groups was similar [angiotensin-converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) (P=0.069), insulin (P=0.978), dipeptidyl peptidase 4 (DPP-4) inhibitor (P=0.943), sulfonylurea (P=0.854), Metformin (P=0.057), and statin (P=0.42)].

Patients with more severe DN had higher Scr (P<0.001), lower eGFR (P<0.001), lower HDL-C (P=0.042), lower FPG (P<0.001), lower HbA1c (P<0.001), higher UACR (P<0.001), higher β2-MG (P<0.001), higher SBP (P<0.001), high-

Table 3. Univariate and stepwise multivariate linear regression for galectin-3 in all DN patients (n=120, without DM)

Univariate model	Std β	95% Confidence Interval	P Value
Age (years)	0.337	0.308 - 0.366	<0.001
Duration of diabetes (years)	0.045	-0.016 - 0.106	0.627
Log (FPG) (mg/dL)	-0.382	-2.266 - 1.502	<0.001
Log (HbA1c) (%)	-0.288	-3.234 - 2.658	0.001
TC (mg/dL)	-0.017	-0.019 - -0.015	0.854
Log (TG) (mg/dL)	0.028	-1.399 - 1.455	0.763
LDL-C (mg/dL)	0.004	-0.004 - 0.012	0.963
HDL-C (mg/dL)	-0.104	-0.129 - -0.079	0.259
Log (Scr) (mg/dL)	0.70	-0.017 - 1.417	<0.001
Log (eGFR) (ml/min/1.73 m^2)	-0.699	-1.303 - -0.095	<0.001
Log (β2-MG) (mg/L)	0.595	0.277 - 0.913	<0.001
Log (UACR) (mg/g)	0.353	0.317 - 0.387	0.002
SBP (mmHg)	0.353	0.339 - 0.367	<0.001
Log (DBP)	0.101	-5.765 - 5.967	0.271
Log (BMI)	-0.070	-5.55 - 5.41	0.453
Stepwise multivariate model	**Std β**	**95% Confidence Interval**	**P Value**
Log (UACR)	0.394	-0.049 - 0.837	0.011
Log (eGFR)	-0.914	-2.398 - 0.567	<0.001

Data of FPG, HbA1c, TG, Scr, eGFR, β2-MG, UACR, DBP and BMI levels showed skewed distribution and therefore were log-transformed. Abbreviatons: FPG, fasting plasma glucose; HbA1c, hemoglobinA1c; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; β2-MG, β2-microglobulin; UACR, urine albumin creatinine ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

er DBP (P=0.028), and higher galectin-3 (P< 0.001). Among all the basic characteristics, it was difficult to match age (P<0.001) and duration of DM (P=0.007), because progression of DN is highly time-dependent.

Correlation between serum galectin-3 levels and clinical variables in patients with DN

Because some variables are clinically associated with renal functions, we performed spearman's correlation analysis to verify the associations between these variables and galectin-3 (**Table 2**). Galectin-3 levels were positively related to Scr, β2-MG, FPG, UACR, age, and SBP, and inversely related to HbA1c and eGFR.

Univariate and multivariate linear regression analyses were used to identify the independent determinants of galectin-3 and the results were summarized in **Table 3**. In univariate model, age, FPG, HbA1c, Scr, eGFR, β2-MG, and UACR were significantly associated with galectin-3. In multivariate model, only eGFR and UACR were independently associated with galectin-3. All of the above mentioned variables were renal function-related or renal function-dependent.

Association of galectin-3 with eGFR and UACR

Since eGFR and UACR were independent determinant of galectin-3, we further investigated the association of galectin-3 with eGFR and UACR by subgroup analysis. Log (galectin-3) was significantly and negatively correlated with log (eGFR) (**Figure 1A**).Compared with control group, the serum level of galectin-3 increased significantly in different stages of DN patients (**Figure 2A**). Even though there were no statistically significant differences among the different stages of CKD, except CKD3 versus CKD4 and CKD4 versus CKD5 (**Figure 2A**), there seemed to be a trend of increasing galectin-3 in the later stages of CKD.

The relationship between serum galectin-3 and UACR is summarized in **Figure 1B**. Log (galectin-3) was significantly and positively correlated with log (UACR), although the correlation was weak. Subgroup analysis showed DN patients with a UACR equal or greater than 300 mg/g (macroalbuminuria) had a higher serum galectin-3 concentration than DN patients with a UACR from 30 to 299 mg/g (microalbuminuria) or a UACR less than 30 mg/g (normal). There was no significant difference of serum galectin-3 levels between microalbuminuria and normal albuminuria groups (**Figure 2B**).

Discussion

Increased serum galectin-3 levels were significantly associated with increased Scr and decreased eGFR levels in type 2 DN patients we examined, even after adjustment for other risk factors. Serum galectin-3 levels were also correlated with UACR, although this correlation was weak. FPG, HbA1c, β2-MG and SBP levels

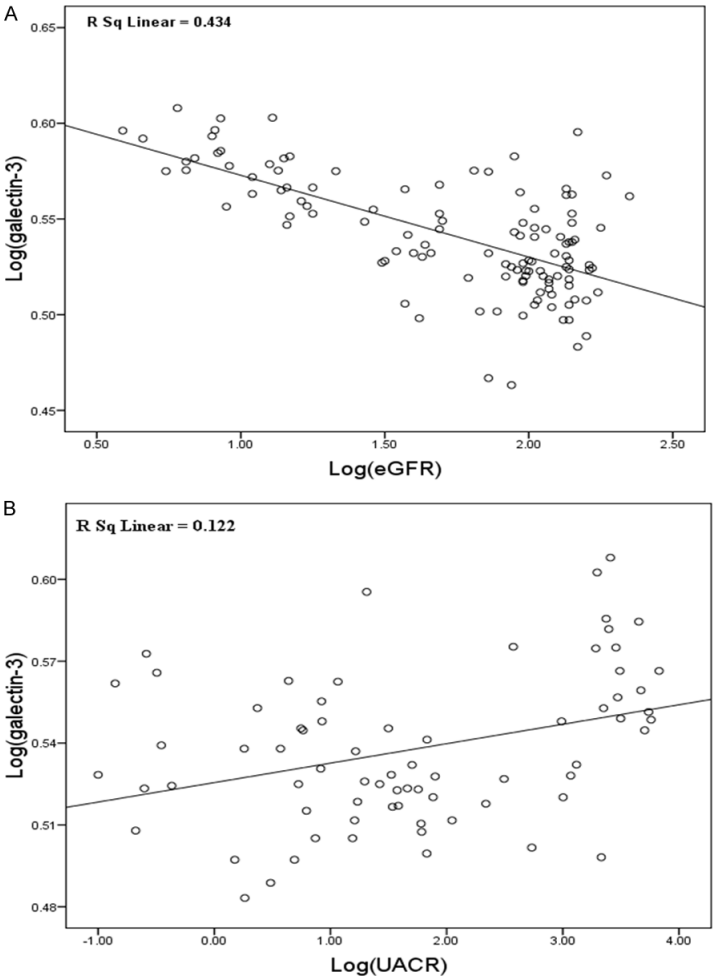

Figure 1. Scatter plots and Spearman's correlation analysis examining the relationship between log (galectin-3) and log (eGFR) or log (UACR) in all patients with DN. A. Correlation between log (galectin-3) and log (eGFR). B. Correlation between log (galectin-3) and log (UACR).

were all associated with serum galectin-3 levels in a univariate linear regression model.

The exact mechanism of elevated serum galectin-3 levels in DN remains unclear. Galectin-3 is strongly expressed in the ureteric bud and in the renal collecting ducts [24] and distal tubules [25]. Elevated levels of galectin-3 have been associated with a number of metabolic disorders and their complications [26], including pre-diabetic conditions, diabetes [10], and microvascular and macrovascular complications found in diabetic patients [27]. Galectin-3 levels have been shown to be increased in patients with a number of diabetes associated complications, including DN [27]. Kikuchi et al reported that the number of galectin-3-positive cells in renal biopsy specimens from patients with diabetes was significantly increased compared with other glomerular diseases. There was also a strongly significant correlation between the number of galectin-3-positive cells in diabetic glomeruli and loss of renal function [28]. This study extends these previous findings in patients with different stages of type 2 DN. We demonstrated that serum galectin-3 levels were significantly and directly associated with eGFR in patients with type 2 DN. When patients were divided according to their CKD status, a trend of higher galectin-3 levels in later CKD stages was observed.

Microalbuminuria is regarded as the gold standard for diagnosing the onset of DN. Hakki et al showed that galectin-3 levels were associated with proteinuria in familial Mediterranean fever (FMF) patients [29], but other studies found that galectin-3 levels were weakly correlated with UACR in patients with diabetes [14, 19]. Patients we evaluated had a significant, but weak, correlation between serum galectin-3 levels and UACR.

It is noteworthy that serum galectin-3 levels were inversely related to HbA1c levels in patients we evaluated. Similar findings have already been reported [30, 31]. It has also been hypothesized that galectin-3 may reduce oxidative stress indirectly by degrading AGEs, leading to lower levels of HbA1c [30]. Although galectin-3 could bind to advanced glycation end-products (AGEs), stimulating their degradation [32], the effect of galectin-3 in reducing HbA1c has not been previously reported. Healthy rats have been shown to not express glomerular or mesangial galectin-3 until they are older [33]. De Boer RA et al also demonstrated the strong relationship between galectin-3 and age in the general population [15]. Consistent with the findings of the previous studies, we confirmed a positive correlation between galectin-3 and age in patients

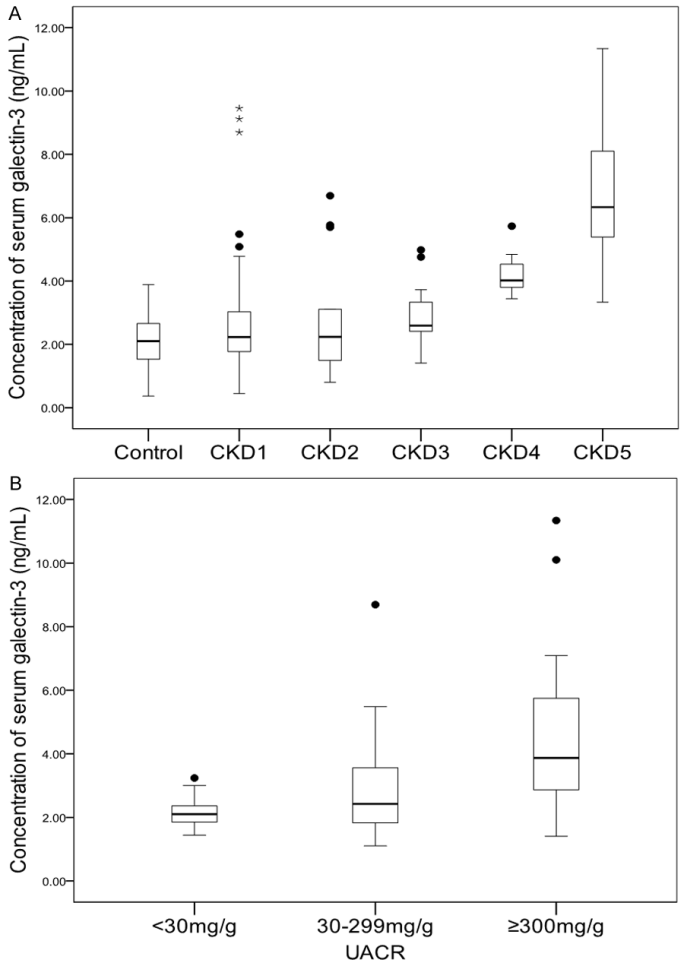

Figure 2. Serum galectin-3 levels correlated with eGFR and UACR. The whisker plot shows the minimum and maximum values, first quartile, median, and third quartile. A. Boxplots showing the distribution of serum galectin-3 concentration by CKD stage. Control versus CKD1, CKD2, CKD3, CKD4, and CKD5 galectin-3 levels had P values of 0.03, 0.0538, 0.006, <0.001, and <0.001, respectively. Galectin-3 levels in different CKD stages in DN patients were also compared; CKD1 versus CKD2 (P=0.579), CKD2 versus CKD3 (P=0.909), CKD3 versus CKD4 (P=0.0052), and CKD4 versus CKD5 (P=0.0066). B. Serum galectin-3 concentrations in DN patients with normal albumin excretion, microalbuminuria, and macroalbuminuria [normal albuminuria group versus microalbuminuria group (P=0.251), normal albuminuria group versus macroalbuminuria group (P<0.001), and microalbuminuria group versus and macroalbuminuria group (P<0.001)].

with type 2 DN. In a previous study, serum galectin-3 levels were found to be associated with SBP in the general population [15]. However, another study observed that serum galectin-3 levels were not related to SBP in T2DM [27]. In our study, we found that serum galectin-3 levels were positively associated with SBP. The reasons for this apparently conflicting finding could be due to differences in research methods and objectives. Although galectin-3 levels correlated with most established risk factors of DN, including age, SBP, HbA1c, FPG, correlation coefficients were generally weak, suggesting that these factors only have a marginal impact on galectin-3 levels.

Several lines of evidence suggest that galectin-3 exerts multiple and sometimes opposing effects in the kidney [34-39]. However, we cannot address whether a similar causal relationship might explain our results. Elevated serum galectin-3 levels may just reflect a compensatory response to renal injury in patients with DN. Stage 1-2 DN is characterized by glomerular hyperfiltration. Serum galectin-3 levels in patients we examined with stage 1-2 DN were significantly lower than those found in the DM group. Further decreases in glomerular filtration rate were associated with further increases in serum galactin-3 levels. It suggested that galectin-3 was, at least partly, cleared by the kidney.

There were several limitations to this study. The research subjects were recruited from a single regional hospital and may not be representative of the entire Chinese type 2 DM and DN population. The sample size was relatively small and could have affected the power of the study to detect small differences. An estimation index based on plasma creatinine levels was used to estimate clearance instead of the true GFR, possibly introducing error into the measurement of renal function. Also, circulating concentration of biomarkers may not reflect those found in the kidney. This is an associative study and cannot be used to determine true casual relationships.

Higher serum galectin-3 concentrations were independently associated to the risk of CKD progression in patients with type 2 DN. These findings suggested that galectin-3 may be a novel predictor for the development of DN.

Longitudinal studies are needed to better define this relationship. The underlying molecular mechanisms should be investigated in future studies.

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Disclosure of conflict of interest

None.

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