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ARTICLE

Galectin-3 is independently associated with progression of nephropathy in type 2 diabetes mellitus

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Abstract

Aims/hypothesis Galectin-3 has been implicated in cardiac and renal fibrosis and serves as a prognostic clinical indicator in heart failure. The aim of the present study was to evaluate whether serum galectin-3 level is associated with progressive kidney disease in type 2 diabetes.

Methods Galectin-3 was measured in baseline samples by ELISA in 1320 participants with type 2 diabetes with eGFR ≥30 ml min^{-1} 1.73 m^{-2}. The primary outcome was defined as doubling of serum creatinine and/or initiation of renal replacement therapy during follow-up. The secondary outcome was progression to macroalbuminuria in individuals with normo- or microalbuminuria at baseline.

Results Serum galectin-3 levels were significantly increased in a random subgroup of 270 type 2 diabetic individuals with eGFR >60 ml min^{-1} 1.73 m^{-2} compared with an age- and sex-matched non-diabetic control group (7.58 ± 2.29 ng/ml vs 6.10 ± 1.91 ng/ml, respectively, $p < 0.01$). In the whole diabetic cohort, after a mean follow-up of 9 years, galectin-3 was independently associated with doubling of serum creatinine (HR 1.19; 95% CI 1.14, 1.24, $p < 0.001$) and incident macroalbuminuria (HR 1.20; 95% CI 1.12, 1.30, $p < 0.001$), even after adjusting for traditional risk factors, baseline eGFR and albuminuria status. Individuals with galectin-3 levels in the highest quartile had a fourfold risk of renal function loss and threefold risk of incident macroalbuminuria.

Conclusions/interpretation Serum galectin-3 was independently associated with progressive renal disease in type 2 diabetes. Further mechanistic studies are warranted to determine whether galectin-3 is simply a disease biomarker or is also a mediator of the development and progression of diabetic nephropathy.

Keywords Diabetic nephropathy · Doubling of creatinine · Galectin-3 · Incident macroalbuminuria · Renal fibrosis · Type 2 diabetes

Abbreviations

ACR	Albumin:creatinine ratio
ACEI	ACE inhibitor
ARB	Angiotensin II receptor blocker
CKD	Chronic kidney disease

Introduction

Galectin-3, a member of the multifunctional galectin family, is ubiquitous expressed [1]. It is localised within the cell and secreted into the extracellular space. Extracellular galectin-3 interacts via its C-terminal carbohydrate-recognition domain with the β-galactoside residues of extracellular matrix and cell surface glycoproteins, whereas interaction of intracellular

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Research in context

What is already known about this subject?

- Galectin-3 is a powerful predictor and prognostic marker of heart failure and mortality
- Galectin-3 is associated with incident kidney disease in population studies

What is the key question?

- Is serum galectin-3 associated with loss of renal function in type 2 diabetes?

What are the new findings?

- Galectin-3 is independently associated with doubling of serum creatinine and incident macroalbuminuria in type 2 diabetes
- Individuals with galectin-3 levels in the highest quartile have a fourfold risk of renal function loss and threefold risk of incident macroalbuminuria

How might this impact on clinical practice in the foreseeable future?

- Galectin-3 may potentially be used as a biomarker to predict progression of nephropathy

galectin-3 occurs via peptide–peptide associations mediated by its N-terminus domain [2]. These structural properties enable galectin-3 to exert multiple functions, and galectin-3 is involved in cell–cell and cell–extracellular matrix adhesion, cell growth and differentiation, apoptosis and angiogenesis [2]. Galectin-3 therefore acts as a broad-spectrum biological response modifier and is involved in tissue fibrosis, tumourigenesis, immunity and the inflammatory response [3–5]. It can exert several and, at times, opposite functions in a number of pathophysiological processes. Recent studies have shown that galectin-3 has a profibrotic effect and is involved in the development and progression of heart failure [6, 7]. Clinical studies have shown that galectin-3 is a powerful predictor and prognostic marker of heart failure and mortality [8, 9]. Furthermore, in the Ludwigshafen Risk and Cardiovascular Health Study, serum galectin-3 concentration was significantly associated with cardiovascular endpoints in individuals with impaired renal function. In individuals receiving dialysis in the German Diabetes Mellitus Dialysis Study, serum galectin-3 was also associated with the combined endpoint of cardiovascular events [10].

Galectin has been implicated in renal disease and galectin-3 is associated with renal fibrosis [11]. The role of galectin-3 in the kidney is complex and appears to be context dependent. Increased galectin-3 expression has been linked to the development of renal fibrosis, but also to attenuation of fibrosis via matrix remodelling [12–14]. Galectin-3 may also play a role in diabetic nephropathy as galectin-3-knockout mice develop accelerated diabetic glomerulopathy [15, 16]. Galectin-3 acts as a clearance receptor for AGE, and galectin-3-deficient mice have marked increases in renal deposition of AGE. Hence, galectin-3 seems to have a beneficial effect in these experimental settings, in contrast to its reported role in renal fibrosis. In clinical studies, serum galectin-3 level is inversely related to kidney function. Data from the Framingham Offspring Study show that elevated plasma levels of galectin-3 are associated with increased risks of rapid decline in glomerular filtration rate and of incident chronic kidney disease (CKD) [17]. A recent community-based population study also demonstrated that higher plasma galectin-3 levels are associated with an elevated risk of developing incident CKD [18]. However, whether the relationship between galectin-3 and CKD is affected by diabetes is not known, and galectin-3 level has not been examined in human diabetic nephropathy. The aim of this study was to determine first, whether there were any changes in serum galectin-3 levels in individuals with type 2 diabetes and normal renal function compared with a non-diabetic control group, and second, whether serum galectin-3 is associated with renal function loss in type 2 diabetes.

Methods

To investigate whether serum galectin-3 is associated with progressive renal disease in people with type 2 diabetic, galectin-3 was measured in baseline samples from a cohort of type 2 diabetic individuals recruited at diabetes clinics from 1996 to 2014 and being prospectively followed up to study the pathogenesis and progression of complications in type 2 diabetes in Chinese people.

Diabetic individuals were invited to participate when they were first seen; major exclusion criteria were non-Chinese descent, type 1 diabetes, malignancy or major illness with limited life expectancy, any change in glucose-lowering or antihypertensive treatment and/or hospitalisation in the preceding 3 months, or unwilling to return for regular follow-up. Serum creatinine was measured annually during follow-up, and more frequently as clinically indicated in those with eGFR

<60 ml min^{-1} 1.73 m^{-2}. For the present study, participants with eGFR <30 ml min^{-1} 1.73 m^{-2} and/or on renal replacement therapy at baseline were excluded. The primary renal outcome was a doubling of serum creatinine from baseline and/or initiation of renal replacement therapy. The secondary outcome was progression to macroalbuminuria from normo- or microalbuminuria at baseline in those participants without doubling of serum creatinine during follow-up. Length of follow-up was calculated as time from baseline examination to the date of doubling of creatinine or the date of progression to macroalbuminuria in those individuals without doubling of serum creatinine, date of dialysis or death, or last follow-up as per the censoring date of 31 January 2017, whichever was earliest. To determine the impact of diabetes on serum galectin-3 concentration, 270 participants with type 2 diabetes and eGFR >60 ml min^{-1} 1.73 m^{-2} were randomly chosen and compared with an age- and sex-matched healthy non-diabetic control group recruited from the community by advertisement. Inclusion criteria were age >40 years, no medical illness and not receiving medication. Informed consent was obtained from all participants and the study was approved by the Ethics Committee of the University of Hong Kong.

Fasting blood samples were taken at baseline for the measurement of HbA_{1c}, creatinine, glucose, lipids and galectin-3, and serum samples were stored at −80°C until assayed. Serum creatinine was measured by the Jaffe method and eGFR was calculated using the Modification of Diet in Renal Disease Study equation. Albuminuria status was determined by urine albumin:creatinine ratio (ACR) from at least two random urine samples collected on two separate occasions within 6 months. Participants were classified as having normoalbuminuria if ACR <3 mg/mmol, microalbuminuria ACR ≥3 mg/mmol and ≤30 mg/mmol, or macroalbuminuria ACR >30 mg/mmol. Serum galectin-3 was measured by ELISA (R&D Systems, Minneapolis, MN, USA) and the intra-assay and inter-assay coefficient of variations were 2.8% and 6.6%, respectively.

Results are expressed as mean ± SD, or as median and interquartile range if the distribution of the data was found to be skewed by Kolmogorov–Smirnov test. Data that were not normally distributed were logarithmically transformed before analyses were made. Comparisons between two groups were done using independent sample *t* test. Pearson's correlation coefficient (*r*) or Spearman's rank correlation coefficient (ρ) was used to test the relationships between variables. Multivariable Cox regression analysis was used to estimate the HRs and 95% CIs for doubling of serum creatinine or progression to macroalbuminuria. The variables included in Cox regression models were those that were statistically significant in univariate analysis or were biologically relevant. Receiver operating characteristic curves were generated for models including traditional risk factors with or without galectin-3. Comparisons between two AUCs for the receiver operating characteristic curves were made by using the DeLong method [19].

Results

A total of 1679 diabetic participants were recruited and 359 individuals were excluded from the analysis (ESM Fig. 1). Individuals who were excluded because of missing data did not significantly differ in terms of their baseline characteristics from those remaining in the study. Out of the 1320 individuals, 78% had eGFR >60 ml min^{-1} 1.73 m^{-2} at baseline and their serum galectin-3 level was significantly lower than those individuals with eGFR between 30 and 60 ml min^{-1} 1.73 m^{-2} (7.70 ± 2.38 vs 9.85 ± 2.90 ng/ml respectively, $p < 0.01$). To determine whether there were changes in serum galectin-3 levels in type 2 diabetic individuals with normal renal function, 270 participants with type 2 diabetes and eGFR >60 ml min^{-1} 1.73 m^{-2} were randomly chosen and compared with a non-diabetic control group. Their clinical characteristics are shown in Table 1. The two groups were matched for age and sex, but the diabetic individuals had significantly higher BMI. Despite eGFR being similar in the

Table 1 Clinical characteristics and serum galectin-3 levels in diabetic individuals with eGFR >60 ml min^{-1} 1.73 m^{-2} and the matched non-diabetic control group

Characteristic	Control *n* = 254	Diabetes *n* = 270
Age (years)	52.4 ± 5.2	52.0 ± 3.2
Men/women (%)	57/43	57/43
Duration of diabetes (years)	-	12.5 ± 6.6
BMI (kg/m^2)	24.2 ± 3.2	26.7 ± 4.6**
Smoker (%)	12.6	10.1
Hypertension (%)	-	67
Normo/micro/ macroalbuminuria (%)	-	75/18/7
Retinopathy (%)	-	30.9
Cardiovascular disease (%)	-	7.7
ACEI/ARB (%)	-	53.8
Lipid-lowering therapy (%)	-	25.7
Systolic BP (mmHg)	121.0 ± 17.2	127.3 ± 19.9**
Diastolic BP (mmHg)	76.2 ± 9.6	77.9 ± 9.2
Fasting glucose (mmol/l)	4.98 ± 0.56	8.61 ± 2.59**
HbA_{1c} (mmol/mol)	38 ± 5.4	69 ± 16.3**
HbA_{1c} (%)	5.66 ± 0.49	8.46 ± 1.49**
Creatinine (μmol/l)	78.2 ± 15.7	76.9 ± 15.0
eGFR (ml min^{-1} 1.73 m^{-2})	82.6 ± 16.0	84.5 ± 16.6
Total cholesterol (mmol/l)	5.25 ± 0.79	4.67 ± 0.92*
Triacylglycerol (mmol/l)	1.10 (0.80–1.40)	1.30 (0.90–1.88)**
LDL-cholesterol (mmol/l)	3.24 ± 0.76	2.73 ± 0.77*
HDL-cholesterol (mmol/l)	1.44 ± 0.36	1.20 ± 0.31*
Galectin-3 (ng/ml)	6.10 ± 1.91	7.58 ± 2.29**

Data are expressed as mean ± SD or median (interquartile range)

* $p < 0.05$ and ** $p < 0.01$ vs non-diabetic control group

two groups, the diabetic individuals had significantly higher serum galectin-3 concentration than the control group. The differences in galectin-3 levels remained significant even after adjusting for BMI. Hence, serum galectin-3 was increased in individuals with type 2 diabetes even when renal function was normal.

During prospective follow-up (mean duration 9 ± 5 years), the primary outcome of doubling of serum creatinine was observed in 270 individuals, of whom 77 individuals required renal replacement therapy (71 with dialysis and six with renal transplantation). In those individuals without doubling of serum creatinine, 139 progressed from normo- or microalbuminuria at baseline to macroalbuminuria. Total mortality in the whole cohort was 8.6%. The baseline clinical characteristics and serum galectin-3 levels are shown in Tables 2 and 3. Serum galectin-3 was significantly increased in the group with doubling of serum creatinine (Table 2) and in those with incident macroalbuminuria (Table 3). In the whole cohort, there were no sex differences in serum galectin-3, and galectin-3 correlated with age ($r = 0.28$, $p < 0.01$), eGFR ($r = -0.37$, $p < 0.01$), urine ACR ($r = 0.16$, $p < 0.01$) and, weakly, with HbA_{1c} ($r = 0.06$, $p = 0.04$). No correlation was seen with BMI, systolic BP or duration of diabetes. There was a significant association between baseline galectin-3 levels and change in eGFR from baseline ($r = -0.29$, $p < 0.01$) (ESM Fig. 2) and in ACR ($\rho = 0.24$, $p < 0.01$) (ESM Fig. 3).

Kaplan–Meier analysis was performed to evaluate the association of serum galectin-3 with the hard renal endpoint of doubling of serum creatinine (Fig. 1). Baseline serum galectin-3 was stratified into quartiles and there was a graded association between increasing quartiles of galectin-3 and renal outcome (logrank test $p < 0.001$). Multivariable Cox regression analysis showed a significant association between serum galectin-3 level (analysed either as quartiles or as a continuous variable) and doubling of serum creatinine. The association remained significant even after adjustment for potential confounders, including baseline eGFR, albuminuria status, age, sex, BMI, duration of diabetes, HbA_{1c}, smoking,

Table 2 Baseline clinical characteristics and serum galectin-3 levels in diabetic individuals with and without doubling of creatinine

Characteristic	Without doubling of creatinine $n = 911$	Doubling of creatinine n = 270
Age (years)	53.9 ± 9.3	58.6 ± 9.8**
Men/women (%)	52/48	58/42
Duration of diabetes (years)	12.4 ± 7.5	12.8 ± 8.1
BMI (kg/m^2)	26.1 ± 4.2	27.0 ± 4.4*
Smoker (%)	10.1	9.6
Hypertension (%)	60.5	80.7
Normo/micro/ macroalbuminuria (%)	64/26/10	18/39/43
Retinopathy (%)	38.4	54.4
Cardiovascular disease (%)	9.9	18.0
ACEI/ARB (%)	45.4	61
Lipid-lowering therapy (%)	28.4	35.2
Systolic BP (mmHg)	129.3 ± 19.6	140.9 ± 23.5**
Diastolic BP (mmHg)	76.4 ± 9.6	78.1 ± 10.6*
Fasting glucose (mmol/l)	8.60 ± 2.76	8.81 ± 3.10
HbA_{1c} (mmol/mol)	69 ± 16.4	72 ± 19.6**
HbA_{1c} (%)	8.44 ± 1.50	8.76 ± 1.79**
Creatinine (μmol/l)	82.5 ± 22.8	107.8 ± 34.2**
eGFR (ml min^{-1} 1.73 m^{-2})	78.6 ± 19.3	60.2 ± 20.3**
Total cholesterol (mmol/l)	4.76 ± 0.97	5.14 ± 1.14**
Triacylglycerol (mmol/l)	1.30 (0.90–1.90)	1.60 (1.10–2.40)*
LDL-cholesterol (mmol/l)	2.82 ± 0.90	3.07 ± 0.98**
HDL-cholesterol (mmol/l)	1.23 ± 0.34	1.16 ± 0.31**
Galectin-3 (ng/ml)	7.15 ± 2.17	10.23 ± 2.93**

Data are expressed as mean ± SD or median (interquartile range)

* $p < 0.05$ and ** $p < 0.01$ vs individuals without doubling of creatinine

Table 3 Baseline clinical characteristics and serum galectin-3 levels in diabetic participants with and without incident macroalbuminuria

Characteristic	No progression to macroalbuminuria $n = 820$	Incident macroalbuminuria $n = 139$
Age (years)	53.5 ± 9.3	56.7 ± 10.6**
Men/women (%)	50.1/49.9	47.5/52.5
Duration of diabetes (years)	12.3 ± 7.5	13.7 ± 7.8*
BMI (kg/m^2)	25.8 ± 40	26.6 ± 4.0
Smoker (%)	10.2	8.3
Hypertension (%)	57.2	77.7
Normo/microalbuminuria (%)	71/29	40/60
Retinopathy (%)	34.3	41.7
Cardiovascular disease (%)	8.8	9.4
ACEI/ARB (%)	41.7	64.0
Lipid-lowering therapy (%)	23.3	27.3
Systolic BP (mmHg)	127.6 ± 18.6	132.7 ± 23.6**
Diastolic BP (mmHg)	76.1 ± 9.5	76.2 ± 8.7
Fasting glucose (mmol/l)	8.57 ± 2.74	8.87 ± 2.78
HbA_{1c} (mmol/mol)	68 ± 16.2	71 ± 18.8
HbA_{1c} (%)	8.41 ± 1.48	8.67 ± 1.72
Creatinine (μmol/l)	80.4 ± 19.5	82.1 ± 22.6
eGFR (ml min^{-1} 1.73 m^{-2})	79.6 ± 17.9	77.9 ± 21.0
Total cholesterol (mmol/l)	4.79 ± 0.98	4.87 ± 1.09
Triacylglycerol (mmol/l)	1.30 (0.90–1.90)	1.40 (1.00–2.00)
LDL-cholesterol (mmol/l)	2.85 ± 0.90	2.86 ± 0.94
HDL-cholesterol (mmol/l)	1.24 ± 0.34	1.24 ± 0.36
Galectin-3 (ng/ml)	7.45 ± 2.17	8.55 ± 2.87**

Data are expressed as mean ± SD or median (interquartile range)

* $p < 0.05$ and ** $p < 0.01$ vs individuals with progression to macroalbuminuria

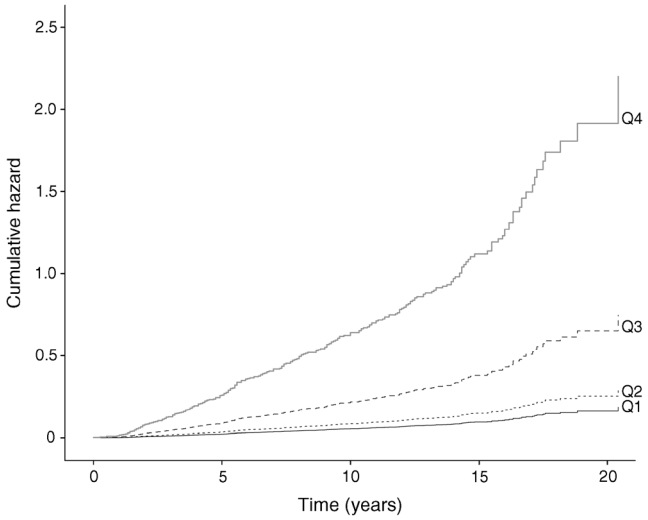

Fig. 1 Kaplan–Meier analysis of primary outcome (doubling of serum creatinine) stratified by quartiles of baseline serum galectin-3. Solid black line, quartile 1; dotted line, quartile 2; dashed line, quartile 3; solid grey line, quartile 4. Q, quartile

systolic BP and ACE inhibitor (ACEI)/angiotensin II receptor blocker (ARB) therapy at baseline. Table 4 shows the results when galectin-3 was analysed as a continuous variable. In the fully adjusted model, when galectin-3 was analysed as quartiles, individuals in the third quartile (>7.68 to ≤9.73 ng/ml, HR 2.00, 95% CI 1.20, 3.34, $p = 0.008$) and the highest quartile of serum galectin-3 (>9.73 ng/ml, HR 4.07, 95% CI 2.47, 6.71, $p < 0.001$) had significantly elevated risk of deterioration of renal function compared with those in the lowest quartile. The AUC for the predictive model comprising traditional risk factors including baseline eGFR, albuminuria status, age, sex, BMI, duration of diabetes, HbA_{1c}, smoking, systolic BP and ACEI/ARB therapy was 0.84 (95% CI 0.82, 0.86). The AUC significantly increased to 0.87 (95% CI 0.85, 0.89) when galectin-3 was added to the predictive model, with the mean difference between the two AUC values being 0.03 (95% CI 0.02, 0.05, $p < 0.01$) (ESM Fig. 4).

To evaluate the association between galectin-3 and progression to macroalbuminuria, 361 individuals with macroalbuminuria at baseline and/or those with doubling of serum creatinine on follow-up were excluded from the analysis. Overall, 139 individuals developed macroalbuminuria during follow-up and there was a significant association between serum galectin-3 and incident macroalbuminuria (HR 1.22, 95% CI 1.14, 1.30, $p < 0.001$). The association remained significant in the fully adjusted model (HR 1.20, 95% CI 1.12, 1.30, $p < 0.001$) (Table 5), and when serum galectin-3 was analysed as quartiles, individuals in the highest quartile had a threefold risk of incident macroalbuminuria (HR 3.23, 95% CI 1.84, 5.68, $p < 0.001$). The AUC for the predictive model comprising baseline eGFR, ACR, age, sex, BMI, duration of diabetes, HbA_{1c}, smoking, systolic BP and ACEI/ARB therapy was 0.70 (95% CI 0.65, 0.75). There was no significant difference in AUC (0.73, 95% CI 0.68, 0.77) when galectin-3 was added to the model (ESM Fig. 5).

Discussion

Recent evidence has shown that circulating galectin-3 is inversely related to renal function [10, 17, 18], and galectin-3 has been implicated in the development of CKD [11]. Galectin-3 plays an important role in the kidney and it

Table 4 Association between serum galectin-3 and doubling of serum creatinine

Characteristic	Model 1		Model 2		Model 3	
	HR (95% CI)	*p* value	Adjusted HR (95% CI)	*p* value	Adjusted HR (95% CI)	*p* value
Galectin-3 (ng/ml)	1.27 (1.23, 1.30)	<0.001	1.23 (1.20, 1.27)	<0.001	1.19 (1.14, 1.24)	<0.001
Age (years)	-	-	1.07 (1.06, 1.09)	<0.001	1.03 (1.01, 1.05)	0.003
Sex (male/female)	-	-	0.52 (0.41, 0.67)	<0.001	0.56 (0.43, 0.73)	<0.001
BMI (kg/m^2)	-	-	1.11 (1.07, 1.14)	<0.001	1.05 (1.02, 1.08)	0.002
Duration of diabetes (years)	-	-	-	-	0.99 (0.98, 1.01)	0.211
Smoker (no/yes)	-	-	-	-	1.08 (0.70, 1.67)	0.717
Systolic BP (mmHg)	-	-	-	-	1.01 (1.00, 1.01)	0.066
HbA_{1c} (mmol/mol)	-	-	-	-	1.02 (1.01, 1.03)	<0.001
Baseline eGFR (ml min^{-1} 1.73 m^{-2})	-	-	-	-	0.97 (0.96, 0.98)	<0.001
Normoalbuminuria	-	-	-	-	Referent	<0.001
Microalbuminuria	-	-	-	-	3.37 (2.36, 4.80)	<0.001
Macroalbuminuria	-			-	7.78 (5.24, 11.57)	<0.001
ACEI/ARB (no/yes)	-	-	-	-	1.11 (0.85, 1.45)	0.450

Model 1: crude model; model 2: adjusted for age, sex and BMI; model 3: further adjusted for duration of diabetes, smoking, systolic BP, HbA_{1c}, baseline eGFR, albuminuria status and ACEI/ARB therapy

Table 5 Association between serum galectin-3 and incident macroalbuminuria

Characteristic	Model 1		Model 2		Model 3	
	HR (95% CI)	*p* value	Adjusted HR (95% CI)	*p* value	Adjusted HR (95% CI)	*p* value
Galectin-3 (ng/ml)	1.22 (1.14, 1.30)	<0.001	1.16 (1.08, 1.25)	<0.001	1.20 (1.12, 1.30)	<0.001
Age (years)	-	-	1.07 (1.04, 1.09)	<0.001	1.05 (1.01, 1.08)	<0.001
Sex (male/female)	-	-	0.69 (0.48, 0.99)	0.043	0.62 (0.43, 0.90)	0.012
BMI (kg/m^2)	-	-	1.10 (1.05, 1.16)	<0.001	1.09 (1.03, 1.14)	0.001
Duration of diabetes (years)	-	-	-	-	1.04 (1.01, 1.07)	0.003
Smoker (no/yes)	-	-	-	-	0.43 (0.17, 1.06)	0.068
Systolic BP (mmHg)	-	-	-	-	1.00 (0.99, 1.01)	0.594
HbA_{1c} (mmol/mol)	-	-	-	-	1.01 (1.00, 1.02)	0.069
Baseline eGFR (ml min^{-1} 1.73 m^{-2})	-	-	-	-	1.01 (1.00, 1.03)	0.037
ACR (mg/mmol)	-	-	-	-	1.03 (1.02, 1.04)	<0.001
ACEI/ARB	-	-	-	-	0.51 (0.34, 0.75)	0.001

Model 1: crude model; model 2: adjusted for age, sex and BMI; model 3: further adjusted for duration of diabetes, smoking, systolic BP, HbA_{1c}, baseline eGFR, ACR and ACEI/ARB therapy

promotes nephrogenesis during development. In the normal adult kidney, it is expressed in the distal renal tubules and collecting ducts, but not in the glomeruli [20]. Galectin-3 is known to participate in inflammatory and fibrotic processes in the heart [6, 7], as well as in the kidney [11–13]. However, in the kidney, galectin-3 can also display pro-regenerative and antioxidative functions [14, 21]. To date, the role of galectin-3 in diabetic nephropathy is still unclear. In renal biopsy specimens from individuals with diabetic nephropathy, Kikuchi et al demonstrated that galectin-3-positive cells were found in the glomeruli and the ratio of galectin-3-positive cells to the total number of macrophages in the tubules was also significantly increased [22]. In this small study, the number of galectin-3-positive cells in glomeruli correlated with urinary protein excretion and with the degree of decline in renal function in the diabetic group. Likewise, we have shown that serum galectin-3 level correlates with ACR and inversely with eGFR in our study. Whether systemic levels of galectin-3 reflect galectin-3 expression in the kidney remains to be determined as galectin-3 can originate from other organs, such as the heart and adipose tissue, and galectin-3 is also cleared, at least in part, not only by the kidney but also by the liver [11, 23].

In people with diabetes and established renal disease receiving dialysis, serum galectin-3 level is associated with cardiovascular outcome [10], but so far there are no available data on the relationship between galectin-3 and the development and progression of diabetic nephropathy. Our study is the first prospective study to investigate the association between galectin-3 and the progression of diabetic nephropathy in people with type 2 diabetes. First, we have shown that serum galectin-3 level is increased in type 2 diabetic individuals without nephropathy (eGFR >60 ml min^{-1} 1.73 m^{-2}), suggesting that the increase in circulating galectin-3 preceded the development of renal impairment. This is in accordance with previous smaller studies showing that individuals with obesity and type 2 diabetes had elevated galectin-3 levels [23, 24]. We further demonstrated that galectin-3 was independently associated with loss of renal function and progression of diabetic nephropathy. Serum galectin-3 was an independent determinant of renal decline even after adjustment for baseline eGFR and albuminuria status. Those individuals with serum galectin-3 level in the highest quartile had a threefold risk of progression to macroalbuminuria and fourfold risk of doubling their serum creatinine. Our results are comparable with those from studies examining the relationship between galectin-3 and incident CKD in population cohorts (the Framingham Offspring Study and the Atherosclerosis Risk in Communities Study) [17, 18]. Both these studies have shown that higher plasma levels of galectin-3 are associated with an increased risk of incident CKD. Incident albuminuria was examined only in the Framingham Offspring Study and no relationship with galectin-3 was found [17]. An independent association between galectin-3 concentration and microalbuminuria has been reported in individuals with chronic heart failure [25].

Although we have shown an independent association between galectin-3 and loss of renal function in diabetic individuals, the role of galectin-3 in the pathogenesis of diabetic nephropathy remains controversial. Galectin-3 is a component of the AGE-receptor complex and is involved in the elimination of these pathogenic compounds [26]. AGEs have been shown to induce the expression of galectin-3 in cultured endothelial cells and within renal tissues in the diabetic milieu [27]. Galectin-3 is therefore involved as part of diabetes-induced adaptive responses and target-organ damage. Animal studies so far suggest that galectin-3 plays a protective

role as an AGE receptor in diabetic nephropathy as galectin-3-knockout mice develop accelerated diabetic glomerulopathy [15, 16]. Galectin-3-deficient mice had marked renal/glomerular AGE accumulation, and this was associated with a pronounced increase in proteinuria, mesangial expansion and matrix gene expression. In rodents with diet-induced obesity, galectin-3 deletion exacerbated systemic inflammation, hyperglycaemia and liver and kidney injury [28]. However, whether galectin-3 participates in tissue repair or promotes kidney damage seems to depend on the underlying aetiology and animal model used. Galectin-3 can exert an opposite role, depending on the stage and degree of tissue inflammation and injury [12, 14]. Pharmacological inhibition of galectin-3 with small-molecule competitive inhibitors prevents hypertensive nephropathy [29]. Targeting galectin-3 has recently been proposed as a new therapeutic approach, especially against kidney fibrosis, regardless of its aetiology [30], and galectin-3 inhibitors are currently being developed. Hence, it is important to determine whether galectin-3 plays a role in protecting from or accelerating the progression of diabetic nephropathy; further mechanistic studies are required.

The main strength of our study is that we have chosen doubling of serum creatinine as the primary endpoint. A doubling of serum creatinine corresponds to a reduction of approximately 57% in eGFR and is a well-established hard renal endpoint to document CKD progression [31].

Our study has several limitations. One of the limitations is the secondary/tertiary nature of our centre. The cases of some of our participants may be more complicated than those of individuals in the general population or primary care and have multiple comorbidities, thus limiting the generalisability of our results. Furthermore, only a small proportion of participants had undergone renal biopsy to ascertain the definitive cause of nephropathy. We cannot completely exclude self-selection bias in the recruitment process, and the large number of missing baseline samples and follow-up data reduced the sample size for analysis and may have also biased our results. There were, however, few differences between those with complete data and those with missing data. We do not have serial measurement of galectin-3 over time and only baseline data were used. Therefore, changes in treatment and progression of disease were not taken into account. As far as we are aware, there are no data on the effect of diabetes medications on galectin-3 levels. Motiwala et al have examined the effect of heart failure medications including ACEI and ARB on galectin-3 levels in individuals with chronic heart failure and found no significant effects [32]. We also cannot address whether the association between galectin-3 levels and changes in renal function is independent of changes in cardiac function as we do not have echocardiographic data or levels of other cardiac biomarkers such as troponins or N-terminal pro-brain natriuretic peptide. Finally, although serum galectin-3 was an independent determinant of renal decline, even after adjustment for baseline albuminuria status, our results showed that albuminuria remains a very strong predictor of progressive diabetic nephropathy, confirming the clinical significance of albuminuria.

In conclusion, we have evaluated the association between serum galectin-3 and the development and progression of diabetic nephropathy. We have shown that serum galectin-3 is independently associated with doubling of serum creatinine as well as incident macroalbuminuria in individuals with type 2 diabetes. Whether galectin-3 is simply a disease biomarker or is also a mediator of the development and progression of diabetic nephropathy warrants further investigation.

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Data availability The summary data that support the findings of this study are available from the corresponding author on reasonable request.

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Contribution statement KCBT designed and oversaw the study, data collection, analysis and drafted the manuscript. CLC contributed to study design and analysed the data. SWMS performed the laboratory assays and analysed the data. YW, JKYL and ACHL recruited the participants and collected clinical data. All authors critically reviewed and approved the final version of the manuscript. KCBT is the guarantor of this work.

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