Endokrynologia Polska
DOI: 10.5603/EP.a2016.0036
Tom/Volume 67; Numer/Number 6/2016
ISSN 0423–104X

Is galectin-3 associated with urinary albumin excretion in type 2 diabetes?

Czy galektyna-3 ma związek z nerkowym wydalaniem albumin w cukrzycy typu 2?

Nihal Yücel[1], Özlem Çakır Madenci[1], Aycan Bölük[1], Lale Köroğlu Dağdelen[1], Yusuf Temel[1], Mehmet Sargın[2], Asuman Orçun[1]

[1]Dr. Lütfi Kırdar Kartal EAH, Biochemistry Department, Turkey
[2]Dr.Lütfi Kırdar Kartal EAH, Diabetes Department, Turkey

Abstract

Introduction: The relationship between galectin-3 and diabetes mellitus or renal function has recently been investigated. In this study, we tried to evaluate the association of galectin-3 in urinary albumin excretion levels in type 2 diabetic patients.

Material and methods: In a group of 137 type 2 diabetes patients, the mean of the last three urinary microalbumin/creatinine ratios and galectin-3 levels were evaluated. The patient group was divided into three subgroups according to their level of albuminuria calculated with urine microalbumin/creatinine ratio.

Results: There was no significant difference between the galectin values of the three subgroups. Significant differences were observed between GFR results of group 1 *vs.* 3 ($p < 0.0001$) and group 2 *vs.* 3 ($p = 0.0006$), and serum creatinine results of group 1 *vs.* 3 ($p = 0.0003$) and group 2 *vs.* 3 ($p < 0.0001$). The three subgroups did not reveal any significant difference concerning the age, BMI, duration of DM, FPG, and HbA_{1c} levels.

Conclusions: We concluded that serum galectin-3 values are not affected by the levels of urinary albumin excretion in DM patients. We could not find any relation between galectin-3 and the parameters of DM such as FPG, HbA1c, and duration of the disease.
(Endokrynol Pol 2016; 67 (6): 580–584)

Key words: *galectin-3; type 2 diabetes mellitus; nephropathy*

Streszczenie

Wstęp: W ostatnim czasie badano związek między galektyną-3 a cukrzycą i czynnością nerek. Autorzy niniejszego badania podjęli próbę oceny związku galektyny-3 z wydalaniem albumin przez nerki u chorych na cukrzycę typu 2.

Materiał i metody: W grupie 137 chorych na cukrzycę typu 2 obliczono średnią ostatnich trzech współczynników mikroalbuminy/ /kreatynina w moczu oraz pomiarów stężeń galektyny-3. Chorych podzielono na trzy podgrupy w zależności od nasilenia albuminuria określonego na podstawie współczynnika mikroalbuminy/kreatynina w moczu.

Wyniki: Nie stwierdzono istotnych różnic pod względem stężeń galektyny między trzema podgrupami. Odnotowano natomiast istotne różnice wartości GFR między grupą 1 i 3 ($p < 0{,}0001$) oraz grupą 2 i 3 ($p = 0{,}0006$) oraz stężeń kreatyniny między grupą 1 i 3 ($p = 0{,}0003$) oraz grupą 2 i 3 ($p < 0{,}0001$). Podgrupy nie różniły się istotnie pod względem wieku, BMI, czasu trwania cukrzycy, FPG ani odsetka HbA_{1c}.

Wnioski: Autorzy wykazali, że stężenia galektyny-3 w surowicy nie zależą od nasilenia wydalania albumin z moczem u chorych na cukrzycę. Nie stwierdzono zależności między stężeniem galektyny-3 a parametrami charakteryzującymi cukrzycę, takimi jak FPG, HbA1c, czas trwania choroby. **(Endokrynol Pol 2016; 67 (6): 580–584)**

Słowa kluczowe: *galektyna-3; cukrzyca typu 2; nefropatia*

Introduction

Nephropathy is one of the major complications of diabetes mellitus. Hyperglycaemia plays the central role in the development of diabetic complications. The biochemical abnormalities include increased polyol and hexosamine pathway activity, oxidative stress, advanced glycated end products (AGE's) formation, and protein kinase C activation [1]. These abnormalities modify the function of the vascular cells and impair the physiological turnover of the vessel wall, resulting in target tissue dysfunction. In the case of nephropathy, the hallmark is an abnormal accumulation of extracellular matrix inside the mesangium [1]. AGE biological effects in this process occur via a receptor-mediated pathway. These effects include downstream signalling and transcriptional events, which play a pivotal role in modulating target tissue injury in diabetes [2].

Galectin-3 is a member of the lectin family of carbohydrate-binding proteins. The molecule includes

Nihal Yücel M.D., Dr. Lütfi Kırdar Kartal EAH, Biochemistry Department, Turkey, e-mail: yucel.nihal@yahoo.com

a C-terminal carbohydrate recognition domain (CRD) with highly conserved residues between family members and an N-terminal domain with a unique short end into an intervening proline-alanine-glycine-tyrosine repeat motif [3]. Galectin-3 localises intra- or extracellularly. Extracellular galectin-3 interacts via the CRD with the β-galactoside residues of extracellular matrix and cell surface glycoproteins. Conversely, interactions of intracellular galectin-3 occur via peptide-peptide associations mediated by its N-terminus domain [2]. These structure and localisation properties of galectin-3 explain its functions in broad-spectrum biological events in several disease conditions [4]. The intracellular effects include pre-mRNA splicing activity, cell control (proliferation, growth and differentiation) and protection from apoptosis. On the other hand, extracellular galectin-3 regulates cell adhesion, takes part in the modulation of immune/inflammatory function, and acts as an AGE receptor [5]. In a study performed on mice, galectin-3 ablation accelerated diabetes-induced glomerulopathy with glomerular lesions, significant albuminuria, and mesangial expansion, which are the functional and structural hallmarks of diabetic glomerulopathy [6]. It is postulated that galectin-3 is upregulated in diabetes and aging because of increased AGE levels; thus it prevents AGE-induced tissue injury [2]. Besides, a more recent study revealed that galectin-3 might be involved in the regulation of glucose homeostasis by acting at the level of adipose tissue and pancreatic islets; it is thus participating in the pathogenesis of obesity and type 2 diabetes [7]. In a clinical study, it was found that in Type 2 diabetes patients, circulating levels of galectin-3 positively correlated with body mass index (BMI) and negatively correlated with glycated haemoglobin [8].

Urinary albumin secretion is the main tool for detection and follow-up of the development of diabetic nephropathy. Taking into consideration the involvement of galectin-3 in diabetes-induced nephropathy, we hypothesise that this molecule could have a relation with urinary albumin excretion. We thereby tried to evaluate the association of galectin-3 with urinary albumin excretion levels in type 2 diabetic patients in this present study.

Material and methods

Study design

A total of 137 Type 2 diabetic patients (57 male, 80 female) followed by the endocrinology department of our hospital were enrolled in the study. According to the follow-up protocol of the diabetes department, microalbumin and creatinine in patients' morning urine samples are analysed every three months. From these, patients who had three consecutive urinary albumin excretion measurements within the last six months were selected. The information about the patients' medical status such as diabetes duration, medication, height, and weight were derived from the patient files. Morning serum and samples were collected. The serum samples were stored at -80ºC until they were tested. The patient group was divided into three subgroups according to their level of albuminuria calculated using the urine microalbumin/creatinine ratio. Group 1 consisted of normoalbuminuric patients who had 0–30 μg/mg albumin/creatinine ratio (n = 45), group 2 consisted microalbuminuric patients who had 30––300 μg/mg albumin/creatinine ratio(n = 47), and group 3 consisted macroalbuminuric patients who had > 300 μg/mg albumin/creatinine ratio (45).

Analytical methodologies

Galectin-3 was measured by micro-ELISA method with Biosensor Human Galectin-3 Platinum Eliza reagent (BMS279/4/BMS279/4TEN). The performance characteristics of the assay given by the manufacturer were as follows; sensitivity 0.29 ng/mL, intra-assay CV 7.5%, and inter-assay CV 5.4%. HbA1c measurement was performed with the NGSP-certified HPLC method on a Variant II Turbo (Bio-Rad, USA). Serum creatinine and fasting glucose (FPG) were tested using an AU5800 analyser (Beckman Coulter, USA) with the reagents of the same manufacturer. The glomerular filtrate rate (GFR) was calculated according to the Cockcroft-Gault formula [9].

Statistical analysis

Descriptive statistics were presented as mean ± standard deviation (SD) and median (2.5–97.5%) as required. The comparison of the groups was done with independent samples Student's t or Mann-Whitney U test. Correlations between clinical variables and the galectin-3 were determined by Pearson's correlation analysis. The Krusker-Wallis test was also performed. Multiple regression analysis was performed to determine the relation between galectin-3 and age, body mass index (BMI), diabetes duration, (GFR), urinary microalbumin excretion, microalbumin/creatinine ratio, (FPG), HbA1c, and serum creatinine. Values of $p < 0.05$ were considered significant. The statistical analyses were performed with Medcalc version 15.2.2 statistical software.

The study protocol was approved by the ethics committee of the hospital.

Results

The descriptive statistics of the total group and three subgroups are presented in Table I. Significant differ-

Table I. *Demographic characteristics and laboratory results summary statistics of the total group and the three subgroups of patients (Mean ± SD or Median [95% CI]) and t-test results between groups*

Tabela I. *Charakterystyka demograficzna i wyniki badań laboratoryjnych w całej badanej grupy i w trzech podgrupach chorych (średnia ± SD lub mediana [95% CI]) oraz wyniki testu t*

	Microalbumin/Creatinine [mg/gr]						
	Group 1 (3–30)	**p value between Group 1 and 2**	**Group 2 (30–> 300)**	**p value between Group 1 and 3**	**Group 3 (> 300)**	**p value between Group 2 and 3**	**Total**
Number (Female/Male)	45 (23/22)		47 (28/19)		45 (29/16)		
Age (year)	58.71 ± 9.97	0.4993	60.23 ± 11.47	0.04*	63.26 ± 10.75	0.1944	60.73 ± 10.84
BMI [kg/m^2]	29 (27 to 30.53)	0.1163	29 (28 to 32)	0.1546	31 (20 to 34)	0.2369	30 (29 to 31)
Duration of DM (year)	13.71 ± 7.79	0.8667	13.98 ± 7.44	0.4286	15.13 ± 9.12	0.5068	14 (12–15)
FPG [mg/dL]	146 (135.46 to 160.77)	0.8458	148 (125 to 162.37)	0.8789	151 (139.46 to 176.62)	0.7249	149 (141–156)
HbA1c (NGSP) (%)	7.37 ± 0.16	0.1705	7.76 ± 1.53	0.1133	7.4 (6.9 to 7.81)	0.8209	7.4 (7.05–7.64)
GFR (Cockcroft-Gault)	82.22 ± 25.40	0.263	76.13 ± 26.44	< 0.0001*	49.4 (41.25 to 58.82)	0.0006*	71.18 ± 29.79
Serum creatinine [mg/dL]	0.83 (0.70 to 0.89)	0.6939	0.83 (0.78 to 89)	0.0003*	1.07 (0.88 to 1.37)	< 0.0001*	0.86 (83–92.35)
Galectin	7.78 ± 2.05	0.8686	7.86 ± 2.83	0.0696	8.68 ± 2.41	0.159	8.09 ± 2.47
Microalbumin/Creatinine [mg/gr] Mean	9.25 (6.49 to 12.91)		67.49 (52.80 to 142.04)		658.32 ± 43.04		67.49 (45.64 to 176.5)
Microalbumin	7.42 (6.22 to 8.60)		60.17 (39.77 to 81.49)		444.94 (325.70 to 511.42)		59.63 (34.07 to 85.33)

*Results with p < 0.05

ences were observed between GFR results of group 1 *vs.* group 3 ($p < 0.0001$) and group 2 *vs.* group 3 ($p = 0.0006$), and serum creatinine results of group 1 *vs.* group 3 ($p = 0.0003$) and the group 2 *vs.* group 3 ($p < 0.0001$). The three subgroups did not reveal any significant difference concerning the age, BMI, duration of DM, FPG, and HbA1c levels. Krusker-Wallis test either did not show any difference between groups with regard to age ($p = 0.09$, BMI ($p = 0.12$), FPG ($p = 0.83$), HbA1c ($p = 0.57$), duration of the disease ($p = 0.76$), and galectin-3 levels ($p = 0.15$). On the other hand, GFR and serum creatinine results of group 3 were significantly different from the results of the other groups ($p < 0.0001$ for both).

Table II displays the multiple regression analysis of galectin with the other parameters. None of the variables was found to be related to galectin-3 according to multiple correlation coefficient or p-values.

The correlation analysis revealed a weak correlation between galectin-3 and microalbumin/creatinine ($r = 0.19$, $p = 0.02$). Galectin-3 was not correlated with any of the other variables (Table III).

Discussion

In addition to the studies on the molecular basis on the subject of galectin-3 and DM and renal disease relation, recently an increasing number of clinical studies have also been performed on this issue [10–12]. In their study on renal biopsy specimens, Kikuchi et al. demonstrated that the number of galectin-3-positive cells in diabetic glomeruli were significantly increased compared with other glomerular diseases [13]. They also noted that this increase could be induced by DM-related substances, such as AGE. Their findings also suggested that galectin-3 expression in glomeruli might be related to DM nephropathy, not DM per se. They could not find any correlation between galectin-3 expression and glycated haemoglobin or duration of DM.

In a study in mice with high-fat diet-induced diabetes, transcriptional upregulation of galectin-3/ /AGE-Receptör3 in the endothelial cells was shown [14]. Since galectin-3 is a component of AGE-receptor complex expressed on the surface of renal cells including endothelial cells, galectin-3 upregulation could be involved in diabetic nephropathy development.

In this present study we intended principally to determine whether galectin-3 could be useful in the evaluation of the development of diabetic nephropathy. Galectin-3 results did not reveal any significant difference between the three groups, as shown in Table I. On the other hand, we found signs of impairment of renal function between the groups; there was a significant difference in GFR and serum creatinine levels in group

Table II. *Multiple regression analysis of galectin with the other parameters*
Tabela II. *Analiza regresji wielokrotnej związku stężenia galektyny z innymi parametrami*

Independent variables	Coefficient	$r_{partial}$	t	P
(Constant)	10.4432			
Age (year)	–0.04405	–0.1392	–1.565	0.1201
BMI [kg/m^2]	0.063	0.1574	1.775	0.0783
Duration of DM (Year)	0.03991	0.1164	1.305	0.1943
GFR	–0.009624	–0.0641	–0.715	0.4758
FPG [mg/dL]	0.003146	0.05275	0.588	0.5575
HbA1c (NGSP) (%)	–0.3157	–0.1288	–1.446	0.1506
Microalbumin/Creatinine [mg/gr]	0.002529	0.09539	1.067	0.288
Serum creatinine [mg/dL]	0.1515	0.02463	0.274	0.7843

Table III. *Correlation coefficients between serum galectin-3 concentrations and other parameters*
Tabela III. *Współczynniki korelacji między stężeniem galektyny-3 w surowicy a innymi parametrami*

	Microalbumin/Creatinine [mg/gr]							
	3–30		30–300		> 300		Total	
Number (Female/Male)	45		47		45		137	
	(r)	P value	(r)	P value	(r)	P value	(r)	P value
Age (year)	–0.14	0.36	–0.1	0.48	–0.007	0.96	–0.05	0.54
BMI [kg/m^2]	0.21	0.16	0.17	0.24	–0.11	0.46	0.16	0.06
Duration of DM (year)	–0.05	0.73	0.1	0.49	0.14	0.34	0.08	0.32
FPG [mg/dL]	–0.11	0.46	0.13	0.36	–0.17	0.25	–0.03	0.72
HbA1c (NGSP) (%)	0.024	0.87	–0.014	0.92	–0.27	0.07	–0.08	0.334
GFR	–0.04	0.79	–0.01	0.92	–0.18	0.24	–0.13	0.12
Serum creatinine [mg/dL]	–0.006	0.96	0.02	0.9	0.2	0.18	0.16	0.06
Microalbumin/Creatinine [mg/gr] Mean	0.009	0.95	0.1	0.49	0.17	0.26	0.19	0.02*
Microalbumin/Creatinine [mg/gr] Last	–0.045	0.77	0.04	0.76	0.072	0.63	0.15	0.07
Microalbumin	–0.11	0.47	0.21	0.16	–0.19	0.21	0.12	0.17

*Results with $p < 0.05$

3 and the other two groups. These findings might be interpreted such that minimal changes in the renal function were not associated with galectin-3 values. In their study, Jin et al. demonstrated a relation between galectin-3 and diabetic complications including nephropathy [15]. They found that galectin-3 levels increased with the number of complications. The difference between that and our study was that they did not separate the complication types as retinopathy, nephropathy, neuropathy; instead, they evaluated them all together. Another difference was that they defined nephropathy using 24-hour urinary albumin excretion, whereas we determined it with the microalbumin/creatinine ratio in spot urine samples. On the other hand, there are also studies that did not find any changes in galectin-3 levels in diabetic nephropathy [8].

One of the results of the study was that galectin-3 levels were weakly correlated with the microalbumin/creatinine ratio in the whole group ($r = 0.19$, $p = 0.02$). A study performed on familial Mediterranean fever (FMF) patients showed that galectin-3 levels were associated with proteinuria [16]. There was a significant difference between galectin-3 levels of patients with and without proteinuria. Although the albuminuria levels of our patients were also significantly different, we could not find this much significance between groups. The mean proteinuria levels of FMF patients were much higher than our patients with macroalbuminuria.

We could not find a relation between galectin-3 and the other parameters in the regression analysis. Serum galectin-3 was not associated with the age, sex, FPG, HbA1c, BMI, or duration of diabetes. Despite the evidence that galectin-3 might participate in the pathogenesis of DM at the level of pancreatic or adipose cells [7], we could not find any relation between galectin-3 and glycaemic status. Our results were also inconsistent with the study of Weigert et al., who found a negative correlation between galectin-3 and glycated haemoglobin [8]. However, there are also reports that supported our results [15]. We think that further clinical studies are required on the subject of galectin-3 and DM disease course, from obesity and insulin resistance to complications.

Conclusions

We can assume that serum galectin-3 values are not associated with levels of urinary albumin excretion in DM patients. We could not find any relation between galectin-3 and the parameters of DM such as FPG, HbA1c, and duration of the disease. We conclude that serum galectin-3 values do not change during minimal renal impairment in the course of DM.

References

1. Pugliese G, Pricci F, Barsotti F et al. Development of diabetic nephropathy in the Milan normotensive strain, but not in the Milan hypertensive strain: possible permissive role of hemodynamics. Kidney Int 2005; 67: 1440–1452.
2. Iacobini C, Amadio L, Oddi G et al. Role of galectin-3 in diabetic nephropathy. J Am Soc Nephrol 2003; 14 (8 Suppl. 3): 264–270.
3. Pugliese G, Iacobini C, Ricci C et al. Galectin-3 in diabetic patients. Clin Chem Lab Med 2014; 52: 1413–1423.
4. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. Biochim Biophys Acta 2006; 1760: 616–635.
5. Pugliese G, Iacobini C, Pesce CM et al. Galectin-3: an emerging all-out player in metabolic disorders and their complications. Glycobiology 2015; 25: 136–150.
6. Pugliese G, Pricci F, Iacobini C et al. Accelerated diabetic glomerulopathy in galectin-3/AGE receptor 3 knockout mice. FASEB J 2001; 15: 2471–2479.
7. Pejnovic NN, Pantic JM, Jovanovic IP et al. Galectin-3 deficiency accelerates high-fat diet-induced obesity and amplifies inflammation in adipose tissue and pancreatic islets.Diabetes 2013; 62: 1932–1944.
8. Weigert J, Neumeier M, Wanninger J et al. Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes. J Clin Endocrinol Metab 2010; 95: 1404–1411.
9. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976; 16: 31–41.
10. Meijers WC, van der Velde AR, Ruifrok WP et al. Renal handling of galectin-3 in the general population, chronic heart failure, and hemodialysis. J Am Heart Assoc 2014; 3: e000962.
11. Ohkura T, Fujioka Y, Nakanishi R et al. Low serum galectin-3 concentrations are associated with insulin resistance in patients with type 2 diabetes mellitus. Diabetol Metab Syndr 2014; 6: 06.
12. Savic J, Zeljkovic A, Bogavac-Stanojevic N et al. Association of small, dense low-density lipoprotein cholesterol and galectin-3 in patients with chronic kidney disease. Scand J Clin Lab Invest 2014; 74: 637–643.
13. Kikuchi Y, Kobayashi S, Hemmi N et al. Galectin-3-positive cell infiltration in human diabetic nephropathy. Nephrol Dial Transplant 2004; 19: 602–607.
14. Darrow AL, Shohet RV, Maresh JG. Transcriptional analysis of the endothelial response to diabetes reveals a role for galectin-3. Physiol Genomics 2011; 43: 1144–1152.
15. Jin QH, Lou YF, Li TL et al. 2013. Serum galectin-3: a risk factor for vascular complications in type 2 diabetes mellitus.Chin Med J (Engl) 2013; 126: 2109–15.
16. Yilmaz H, Inan O, Darcin T et al. Serum galectin-3 levels were associated with proteinuria in patients wih Familial Mediterranean Fever. Clin Exp Nephrol 2015; 19: 436–42.