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Correlation of serum galectin-3 level with renal volume and function in adult polycystic kidney disease

Sultan Ozkurt[1] · Ibrahim Dogan[2] · Oguzhan Ozcan[3] · Nurdan Fidan[4] · Ilter Bozaci[1] · Behice Yilmaz[5] · Muzaffer Bilgin[6]

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Abstract

Purpose The decrease in kidney functions in autosomal dominant polycystic kidney disease (ADPKD) is strongly correlated with the severity and growth of kidney cysts. Total kidney volume (TKV) was shown to be an early marker of the severity of the disease and a predictor of reduction in kidney functions. New treatment approaches for ADPKD have led to a need for easily applicable strong biomarkers predicting progression of the disease. The profibrotic mediator of galectin-3 (Gal-3) is linked to development of renal fibrosis.

Methods The study included 74 patients with ADPKD diagnosis and 40 healthy controls. The TKV of patients was calculated using the manual tracing method on MR images. The serum Gal-3 levels of patient and healthy control groups were measured with the ELISA method. The correlations between serum Gal-3 value with TKV and kidney function were assessed in patients.

Results As the stage of chronic kidney disease (CKD) increased, serum Gal-3 and TKV values increased ($p < 0.001$, $p = 0.049$, respectively). Correlation analysis found a negative relationship between serum Gal-3 levels and eGFR (r: -0.515, $p < 0.001$); however, there was no relationship between serum Gal-3 and TKV ($r = 0.112$, $p = 0.344$). Linear regression analysis showed the major parameter affecting Gal-3 was eGFR ($p = 0.016$).

Conclusions In our study, we showed that renal impairment is an important determinant of Gal-3, and there is no correlation of Gal-3 and TKV in ADPKD. As a result, there is an urgent clinical need for new biomarkers to identify individuals with the chance of treatment in the early stage among ADPKD patients.

Keywords Autosomal dominant polycystic kidney disease · Fibrosis · Galectin-3 · Total kidney volume

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disorder comprising of nearly 10% of all patients with end-stage renal disease in Europe [1]. Though cysts develop from birth, due to compensatory hyperfiltration of the remaining functional nephrons, the glomerular filtration rate (GFR) is probably preserved for decades linked to the mutations present [2]. PKD1 mutations, especially patients with truncate mutations, have more serious clinical progression compared to those with PKD2 mutations and the median age of ESRD onset is 58 and 79 years, respectively [3]. The growing cysts compress healthy kidney tissue, causing progressive reduction in kidney functions and most patients require renal replacement treatment in the end. For ADPKD patients, total kidney volume (TKV) is an early marker of the severity of

✉ Sultan Ozkurt
dr.s.guvenir@hotmail.com

1 Department of Nephrology, Faculty of Medicine, Eskisehir Osmangazi University, Büyükdere, 26040 Odunpazarı, Eskisehir, Turkey

2 Department of Nephrology, Faculty of Medicine, Hitit University, Çorum, Turkey

3 Department of Biochemistry, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey

4 Department of Radiology, Faculty of Medicine, Hitit University, Çorum, Turkey

5 Department of Radiology, Haseki Education and Research Hospital, Istanbul, Turkey

6 Department of Biostatistics, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey

the disease and is shown to be a determinant of the reduction in kidney functions [4].

Until recently, ADPKD treatment was performed symptomatically without affecting cyst formation and as a result renal expansion [5]. The TEMPO 3:4 study in 2012 showed that the vasopressin V2 receptor antagonist tolvaptan significantly reduced the annual increase in TKV [6]. The increasing use of TKV as a marker of ADPKD progression has brought the question of how TKV can best be measured to the agenda and it appears magnetic resonance (MR)-based imaging methods are ideal for TKV measurement [7].

The gold standard method to assess TKV is the manual tracing method. For this, computed tomography or magnetic resonance images are used and the boundaries of the kidney are manually traced on each slice using special software. Kidney volume was obtained by taking the area of each kidney manually determined on sequential slices, multiplying by the slice thickness and then adding all the slice volumes together. This method has high repeatability and provides accurate kidney volume measurements but is laborious and takes time, which limits its use to clinical studies [8].

Medications slowing the growth of cysts have shown the greatest benefit in the early disease stage when cysts have not yet caused irreversible injury [9]. However, time-consuming and expensive imaging methods such as MR are not routinely recommended for TKV monitoring to determine disease prognosis and early treatment [8]. As a result, there is a need for a strong biomarker predicting the progression of disease.

As cysts grow, they block the tubules of both local and higher up nephron units, disrupt the venous system and as a result, they endanger renal blood flow, causing inflammation and interstitial fibrosis [10].

Galectin-3 (Gal-3) is a b-galactoside-binding lectin released by activated macrophages and has attracted attention as a new biomarker reflecting inflammation and tissue fibrosis [11, 12]. To understand how the renal fibrosis caused by increasing cyst volume in ADPKD affects Gal-3 levels and whether Gal-3 is correlated with TKV or not, this study used the manual tracing method on MR images of ADPKD kidneys in different stages with the aim of assessing the correlation between renal function and Gal-3 with TKV.

Materials and methods

Patients, sample collection and biochemical measurements: The study included 74 patients with ADPKD diagnosis aged from 18 to 65 years attending Hitit University, Faculty of Medicine Nephrology clinic, and 40 healthy controls. The required sample size was determined using power analysis. Patients were divided into three groups based on estimated glomerular filtration rates (eGFR). The first group included patients with eGFR above 60 ml/min/1.73 m^2, the second group included patients with eGFR from 30 to 60 ml/min/1.73 m^2 and the third group included patients with eGFR below 30 ml/min/1.73 m^2. eGFR was calculated using the CKD EPI formula [13]. Fasting blood samples were obtained by venipuncture from patients and healthy controls. All samples were centrifuged at 1500*g* for 10 min at 4 °C. Serum samples were aliquoted and stored at − 80 °C until the analysis.

Serum galactin levels were assayed by ELISA method (Thermo Scientific Multiskan GO, Finland) using commercially available galactin ELISA Kits. The assay ranges for the galactin kit were 0.156–10 ng/ml, the intra- and inter-assay coefficients of variance (CV%) were 10% and 12%, respectively. Routine tests were measured with standard methods. The majority of patients were hypertensive and hypertensive patients treated with angiotensin-converting enzyme inhibitor, angiotensin receptor blockers, calcium channel blockers or combined treatments.

Patients had total kidney volume calculated with the manual tracing method on MR images taken without contrast [8].

MRI protocol and image analysis

MRI data were acquired with 1.5 T MR (Optima MR450w, GE Healthcare, Milwaukee WI, USA) using 32-channel phase-arrayed body coils. The imaging protocol included unenhanced sequences only. Field of view was maintained between 42 × 42 cm with a matrix size of 256 × 256 and 4 mm slice thickness, no interslice gap. The volumes of the right and left kidneys were separately measured by the same radiologist using the manual boundary tracing method. On coronal images, the kidney boundaries were manually drawn one-by-one on each slice. Kidney volume was obtained by multiplying the total area drawn manually for each kidney by the slice thickness and automatically adding the volumes for all slices. The right and left kidney volumes were added to calculate TKV.

Patients with inflammatory situations that may affect serum Gal-3 levels or congestive heart failure causing fibrosis, rheumatological or systematic inflammatory diseases, with acute infection, diagnosed malignancy, liver disease or diabetes were excluded from the study. Assessment of laboratory and radiological data, statistical analysis and writing the results were performed by Eskişehir Osmangazi University, Faculty of Medicine Nephrology department.

Statistical analysis

Continuous data are given as mean ± standard deviation. Categorical data are given as percentages (%). The Shapiro–Wilk's test was used to investigate the normal distribution of the data. One-way ANOVA was used for the

comparison of normal distribution groups and for comparisons with three or more groups. The Kruskal–Wallis *H* test was used for the comparison of groups without normal distribution, and for groups with three or more groups. The Spearman correlation coefficients were calculated for variables determining the direction and magnitude of the relationship (correlation) between the variables, and for variables that did not show normal distribution. Pearson Chi-square and Pearson exact Chi-square analyses were used for the analysis of the generated cross-tables. Linear regression analysis was used to determine the relationships between variables and multiple relationships. Correction terms are used in linear regression analysis. The analysis was implemented using IBM SPSS Statistics 21.0 (IBM Corp. released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp.). For statistical significance, $p < 0.05$ was considered the criterion.

Results

The study included 74 patients. There were 39 patients in group 1, 17 in group 2 and 18 in group 3 with 40 in group 4 (control group). The mean ages in groups 1 and 4 were statistically significantly low compared to the mean ages in groups 2 and 3. Table 1 shows the clinical and laboratory characteristics of patients. As the CKD stages increased, TKV was identified to increase, but this only reached statistical significance for the TKV of group 3 compared to group 1 ($p = 0.015$). There were statistically insignificant differences between the other groups (Table 2). Serum

Table 1 Participant characteristics

	Group 1 ($n = 39$)	Group 2 ($n = 18$)	Group 3 ($n = 17$)	Group 4 ($n = 40$)	*p* value	Multiple comparison		
Age (years)	43.03 ± 13.6	58.39 ± 13.5	58.65 ± 10.98	46.65 ± 8.58	< 0.001*	1	2	< 0.001
							3	< 0.001
							4	0.169
						2	3	0.948
							4	0.001
						3	4	0.001
Gender (male/female)	15/24	9/9	9/8	20/20	0.663**			
Body mass index (kg/m^2)	28.35 ± 4.2	27.97 ± 3.08	28.95 ± 6.06	27.28 ± 3.61	0.505*			
Smoking (%)	3 (7.7%)	3 (16.7%)	2 (11.8%)	1 (2.5%)	0.264***			
Fasting glucose (mg/dl)	100.5 ± 24.4	98.2 ± 18.61	100.8 ± 22	92.4 ± 16.6	0.293*			
Plasma creatinine (mg/dl)	0.83 ± 0.22	1.57 ± 0.33	4.45 ± 1.81	0.78 ± 0.16	< 0.001****	1	2	< 0.001
							3	< 0.001
							4	0.728
						2	3	< 0.001
							4	< 0.001
						3	4	< 0.001
eGFR (ml/min/1.73 m^2)	95.85 ± 24.2	43.11 ± 9.2	14.12 ± 5.9	102.48 ± 12.4	< 0.001*	1	2	< 0.001
							3	< 0.001
							4	0.078
						2	3	< 0.001
							4	< 0.001
						3	4	< 0.001
Spot urine protein/Cr (g/day)	0.16 ± 0.14	0.63 ± 0.66	1.45 ± 0.86	0.07 ± 0.03	< 0.001****	1	2	0.079
							3	< 0.001
							4	< 0.001
						2	3	0.352
							4	< 0.001
						3	4	< 0.001

eGFR estimated glomerular filtration rate, *Cr* creatinine

*One-way ANOVA, **Pearson Chi-square test, ***Pearson exact Chi-square test, ****Kruskal–Wallis *H* test

Table 2 Participant TKV and gal-3 values

	n	Mean ± SD	*p**	Multiple comparisons		
TKV (cm^3)						
Group 1	39	1247.84 ± 777.59	0.049	1	2	0.577
Group 2	18	1401.44 ± 998.88			3	0.015
Group 3	17	1941.94 ± 1265.25		2	3	0.101
Gal-3						
Group 1	39	2.86 ± 1.91	< 0.001	1	2	0.030
Group 2	18	4.01 ± 2.72			3	0.009
Group 3	17	4.28 ± 2.40			4	< 0.001
Group 4	40	1.05 ± 0.56		2	3	0.670
					4	< 0.001
				3	4	< 0.001

TKV total kidney volume, *Gal-3* galectin-3

*Kruskal–Wallis *H* test

Gal-3 level was lowest in the control group. As CKD stage increased, there was a significant level of increase in serum Gal-3 levels. This difference was only statistically significant for group 3 and group 2 (Table 2). Correlation analysis found a positive relationship between serum Gal-3 level and serum creatinine, and spot urine protein/Cr ratio and a negative relationship with eGFR (r: 0.502, $p < 0.001$, r: 0.541, p: < 0.001, r: $-$ 0.515, $p < 0.001$, respectively); there was no relationship found with TKV (r: 0.112, $p = 0.344$). After corrections for age between the groups, there was no correlation observed between TKV and Gal-3 ($p = 0.145$). Linear regression analysis found the major parameters affecting TKV were age, eGFR and proteinuria ($p = 0.029$, $p \leq 0.0001$, $p = 0.006$, respectively) and the major parameter affecting Gal-3 was eGFR ($p = 0.016$). Figure 1 shows the Gal-3 distribution in the groups, while Fig. 2 shows the distribution of TKV values in the groups.

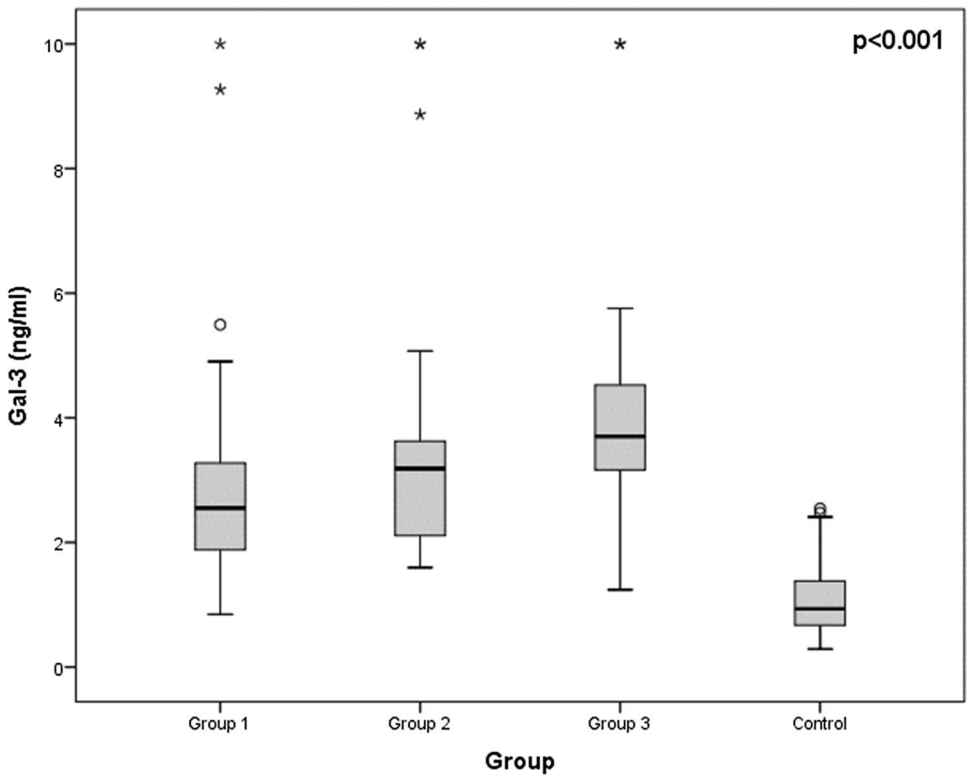

Fig. 1 Gal-3 distribution in the groups

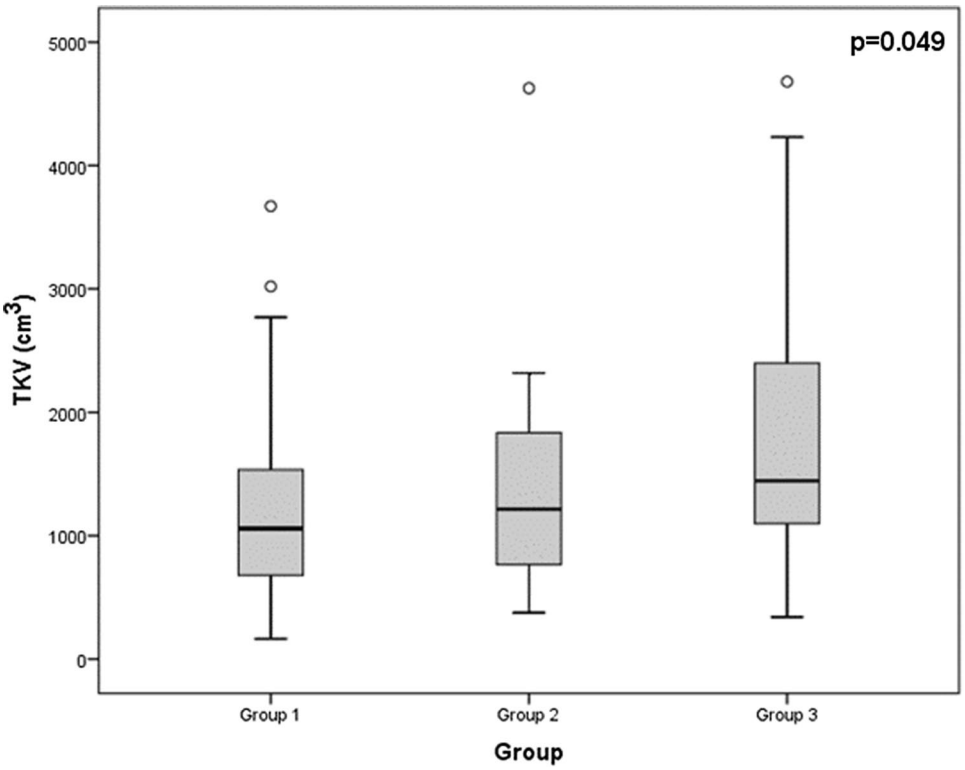

Fig. 2 TKV distribution in the groups

Discussion

Gal-3 is upregulated in a variety of inflammatory diseases affecting the liver, lungs and kidneys, and was also shown to play an important role in regulating profibrotic pathways in the heart [14–16]. Therefore, clinical data support the idea that serum Gal-3 levels reflect myocardial fibrosis [17]. Gal-3 is described as a newly revealed biomarker for prognosis and risk stratification of patients with acute and chronic heart failure (HF) [15, 18]. However, there are studies indicating a contradiction about whether high Gal-3 levels in HF are due to cardiac fibrosis or due to renal failure [19, 20].

As the cyst volume increases in ADPKD, there is a reduction in renal perfusion linked to the compression of normal renal tissue and as a result it is a progressive disease leading to interstitial fibrosis [10]. In our study, we assessed whether renal fibrosis linked to increasing renal volume affects serum Gal-3 levels. To the best of our knowledge, this is the first study assessing TKV and Gal-3 levels in ADPKD. In our study, we found that as CKD stage increased, serum Gal-3 level increased; however, we did not show a correlation between TKV and Gal-3 levels. Linear regression analysis found the most significant factor affecting serum Gal-3 levels was eGFR.

Grupper et al. [19] showed that in HF patients, the majority of patients had high Gal-3 levels after heart transplantation, and that elevated Gal-3 was associated with renal function disorder. Meijers et al. [20] found that in HF patients, despite high plasma Gal-3 levels, the galectin-3 level in urine did not increase and they proposed that this situation may be due to impaired renal handling of galectin-3 in patients with HF. Gopal et al. [21] investigated the relationship between eGFR and Gal-3 in HF patients with both preserved and reduced ejection fractions. They found a strong inverse relationship between Gal-3 levels and renal function in HF patients, and proposed that Gal-3 may be a major determinant of renal function rather than HF [21]. In our study, we showed a strong negative correlation between serum Gal-3 level and eGFR, complying with these studies. Additionally, the Framingham heart study reported that Gal-3 predicted new onset HF and a new article by the same group reports that plasma Gal-3 changed before the development of renal disease. As a result, Gal-3 is not just a disease marker but is also proposed to contribute to the development and progression of heart and kidney diseases [22]. As a result, it is important to understand how Gal-3 is regulated and handled because continuous increases may not only be a marker of outcomes but may continue disease progression.

The role of galectin-3 in progression of polycystic kidney disease is not fully determined. Gal-3 is a key regulator of terminal differentiation and growth of collecting ducts in the kidney [23, 24], and is proposed to play a role in pathogenesis of autosomal recessive polycystic kidney disease (ARPKD) related to hepatorenal congenital fibrocystic syndromes [25]. The correlation between Gal-3 expression and development of kidney cysts is emphasized as due to the

role of gal-3 in epithelial growth and differentiation [26]. A new study shows that abnormally increasing STAT 6 activation with the IL-13 signal in PKD is caused by the excessive expression of four proteins such as periostin, IL-24 and pIgR and including gal-3. These proteins are excessively expressed in polycystic kidneys caused by a variety of genetic defects; this IL-13 signal and then STAT6 activation may be regulated by coordinated expression of a range of genes with the general feature of accompanying renal cyst growth [27].

It is still uncertain as to whether elevated Gal-3 is a result of reduced renal functions or if high levels of Gal-3 cause greater loss of kidney functions. In our study, the lack of correlation between TKV and Gal-3 and the lack of change in this result after age correction between the groups leads to the consideration that Gal-3 levels may be elevated as a result of renal function disorder rather than due to increased fibrosis.

There are many potential mechanisms for increasing Gal-3 levels in the presence of renal failure. The first is that Gal-3 clearance (molecular weight—30 kDa) occurs from the kidneys and thus it is possibly a marker of reduced kidney function. The second is that there may be increased Gal-3 production from the kidney [28, 29]. Third, it is possible that Gal-3 is produced in the kidney and may exhibit profibrotic effects resulting in impaired renal function. Additionally, evidence shows that Gal-3 may protect the kidney from ischemia/reperfusion and CKD [30, 31]. Finally, increased Gal-3 in renal failure may possibly reflect production from organs other than the heart and kidneys in systemic inflammation situations that may occur in both HF and CKD [32–34].

Our study is the first to show the relationship between TKV and Gal-3 in ADPKD, and is important because TKV was studied with the gold standard method. Additionally, our study has some limitations. The first is the low number of patients, the second is the lack of TKV monitoring due to the cross-sectional design of the study and the third is that mutation analysis was not performed. In conclusion, our study shows that plasma Gal-3 is an important determinant of renal disorder and that there is no correlation between Gal-3 and TKV in ADPKD. As a result, there is an urgent clinical need for definition of new biomarkers to identify individuals with chance of treatment at the earliest stage among ADPKD patients.

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Compliance with ethical standards

Conflict of interest There are no declared conflicts of interest in this study.

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