Clin. Lab. 2017;63:XXX-XXX

ORIGINAL ARTICLE

Fibulin Levels in Autosomal Dominant Polycystic Kidney Disease and Its Relationship to Arterial Stiffness

A. Inci [1], O. Akin [1], HY. Ellidag [2], E. Eren [2], S. Dolu [3], F. Sari [4]

[1] *Antalya Training and Research Hospital, Internal Medicine, Division of Nephrology, Antalya, Turkey*
[2] *Antalya Training and Research Hospital, Internal Medicine, Division of Biochemistry, Antalya, Turkey*
[3] *Antalya Training and Research Hospital, Internal Medicine, Division of Internal Medicine, Antalya, Turkey*
[4] *Akdeniz University, Faculty of Medicine, Internal Medicine, Division of Nephrology, Antalya, Turkey*

SUMMARY

Background: **The objective of the present study was to evaluate fibulin 1 levels in different stages of patients with autosomal dominant polycystic kidney disease (ADPKD) and investigate possible connections between fibulin-1 and arterial stiffness.**
Methods: **For this cross-sectional study, we included 74 patients with ADPKD (mean age, 50.92 ± 15.70 years) and 32 healthy controls (mean age, 49.53 ± 7.32 years). Patients with ADPKD were classified based on CKD epidemiology collaboration (CKD-EPI) equation assessments of estimated glomerular filtration rate (eGFR). Blood levels of fibulin 1 and creatinine levels were analyzed. We measured brachial artery PWV (baPWV), augmentation index (AIx), and pulse pressure (PP) for the assessment of arterial stiffness and systolic and diastolic blood pressures (SBP and DBP, respectively).**
Results: **Fibulin 1 was significantly higher in the patient group ($p < 0.001$). SBP, DBP, MAP, PP, and baPWV levels were also significantly higher in the patient group. A statistically significant positive correlation was found between fibulin 1 and creatinine ($r = 0.377$, $p = 0.001$). No significant correlation was found between the fibulin 1 levels and age, SBP, DBP, MAP, baPWV, and AIx.**
Conclusions: **Plasma concentrations of fibulin 1 increased in patients with ADPKD. Arterial stiffness measured by baPWV increased in patients with ADPKD, but it was not related to fibulin 1 levels.**
(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2017.170617)

Correspondence:
A. Inci
Antalya Training and Research Hospital
Internal Medicine
Division of Nephrology
Antalya
Turkey
Phone: +90 5053557358
Fax: +90 242 2494400
Email: aycainci2004@hotmail.com

Manuscript accepted June 30, 2017

KEY WORDS

fibulin, arterial stiffness, autosomal dominant polycystic kidney disease

LIST OF ABBREVIATIONS

ADPKD - autosomal dominant polycystic kidney disease
ESRD - end-stage renal disease
CVD - cardiovascular disease
PWV - pulse wave velocity
CKD - chronic kidney disease
CKD-EPI - CKD epidemiology collaboration
eGFR - estimated glomerular filtration rate
baPWV - artery PWV

AIx - augmentation index
PP - pulse pressure
SBP - systolic blood pressure
DBP - diastolic blood pressure
ARB - angiotensin receptor blockers
ACEi - angiotensin-converting enzyme inhibitors

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a systemic, hereditary disease with cystic changes in both kidneys, occurring in 1 in every 400 to 1,000 live births [1,2]. ADPKD is responsible for 7% - 10% of cases of end-stage renal disease (ESRD) [3]. Vascular dysfunction has been shown to develop from early stages in ADPKD, and cardiovascular disease (CVD) is the most common cause of morbidity and mortality [4,5]. Arterial stiffness is an important indicator of atherosclerosis and cardiovascular events. Increased arterial stiffness causes systolic hypertension, left ventricular hypertrophy, and coronary perfusion impairment, leading to increased cardiovascular risk. Pulse wave velocity (PWV) is frequently used as an arterial stiffness indicator in chronic kidney disease (CKD) [6].
Fibulin is an extracellular matrix glycoprotein and has an active role in the proliferation and migration of smooth muscle cells and endothelial cells [7]. Fibulin 1 acts in conjunction with fibrinogen and plays a role in the etiology and progression of CVD, particularly with respect to thrombotic aspects of atherosclerosis [8]. Cangemi et al. reported that fibulin 1 accumulates in the arterial wall and plasma of patients with type 2 diabetes and is associated with arterial extracellular matrix changes in type 2 diabetes [9]. Increased plasma fibulin 1 levels were also observed in patients with CKD [10], and a multiplex proteomic study showed that plasma fibulin 1 could be a marker for renal impairment in four types of kidney disorders: glomerulonephritis, diabetic nephropathy, obstructive uropathy, and analgesic abuse [11]. Extracellular matrix alterations have been suggested to be part of the early events occurring in ADPKD [12]. The objective of the present study was to evaluate fibulin 1 levels in different stages of patients with ADPKD and investigate possible connections between fibulin-1 and arterial stiffness.

MATERIALS AND METHODS

For this cross-sectional study, we included 74 patients with ADPKD (mean age: 50.92 ± 15.70 years) from the outpatient clinic of the Antalya Training and Research Hospital, Nephrology Unit, between January and June 2014. The control group included 32 healthy individuals (mean age, 49.53 ± 7.32 years). Patients aged < 18 years, pregnant women, those with hepatic diseases, diabetes mellitus, other kidney diseases, clinically apparent infections, active malignancy, and glomerular filtration rate < 15 mL/minute were excluded from the study. This study was conducted according to the Declaration of Helsinki and the guidelines of Good Clinical Practice and was approved by the local ethics committee. All patients provided written informed consent. Patients with ADPKD were classified based on CKD epidemiology collaboration (CKD-EPI) equation assessments of estimated glomerular filtration rate (eGFR) as CKD Stage 1 (≥ 90 mL/minute/1.73 m^2), CKD Stage 2 (60 - 89 mL/minute/1.73 m^2), CKD Stage 3 (30 - 59 mL/minute/1.73 m^2), and CKD Stage 4 (15 - 29 mL/minute/1.73 m^2).
Blood and urine samples were collected in the morning after an 8-h fast. The serum was stored at -80°C. Blood levels of fibulin 1 and creatinine levels were analyzed. Serum fibulin 1 levels were measured using a commercially available ELISA kit (YH Biosearch, Shanghai, China). The inter- and intra-assay coefficients of variation were both < 10%. The detection range of the fibulin kit assay was 5 - 1,500 ng/mL, and the sensitivity is 2.51 ng/mL. The assays used the quantitative sandwich enzyme immunoassay technique. To avoid variation within an assay, measurements were performed in duplicate and simultaneously using the same ELISA kit. PWV and blood pressure were analyzed using the Mobil-O-Graph 24-h PWA monitor (I.E.M. Industrielle Entwicklung Medizintechnik und Vertriebsgesellschaft mbH, Stolberg, Germany). This device's built-in algorithms were used for the central aortic and brachial blood pressure measurements by combining the pulse wave analysis. We measured brachial artery PWV (baPWV), augmentation index (AIx), and pulse pressure (PP) for the assessment of arterial stiffness and systolic and diastolic blood pressures (SBP and DBP, respectively) in the supine position after 5-minutes bed rest. Continuous variables are presented as mean ± standard deviation and categorical variables as percentages. The Kolmogorov-Smirnov test was used to verify the normality of the distributions of continuous variables. For the statistical analysis of the clinical data between two groups, we used unpaired *t*-tests for parametric data and the Mann-Whitney *U* test for nonparametric data; one-way analysis of variance or the Kruskal–Wallis test was used to evaluate comparisons between three or more groups. A Bonferroni correction was applied in post-hoc analyses. Correlations were assessed with Pearson's or Spearman's correlation coefficients, and the chi-square test was used for categorical variables. The analyses were performed with PASW 18 software (SPSS/IBM, Chicago, IL, USA), and two-tailed p-values of < 0.05 were considered statistically significant.

RESULTS

The baseline characteristics of the patients with ADPKD (n = 74) and controls (n = 32) are shown in Table 1. Age and gender were not different between the patient group and healthy controls. Creatinine level was

Table 1. Baseline characteristics of the patients with ADPKD and the controls.

Parameter	ADPKD (n = 74)	Control (n = 32)	p-values
Age (years)	50.92 ± 15.70	49.53 ± 7.32	0.622
Gender (F/M)	33/41	12/20	0.498
Creatinine (mg/dL)	1.69 ± 0.94	0.89 ± 0.13	< 0.001
eGFR (mL/min/1.73 m^2)	57.59 ± 33.34	90.02 ± 20.39	< 0.001
Disease prevalence, n (%)			
Hypertension	46 (62.4%)	-	
Hyperlipidemia	8 (10.8%)	-	
Coronary artery disease	6 (8.1%)	-	
Peripheral artery disease	3 (4.05%)	-	
Stroke	8 (10.8%)	-	
Medications, n (%)			
ACEi	18 (24.3%)	-	
ARB	22 (29.7%)	-	
Fibulin (ng/mL)	301.59 ± 255.35	130.85 ± 176.96	< 0.001
SBP (mmHg)	128.17 ± 15.18	115.97 ± 13.21	< 0.001
DBP (mmHg)	84.35 ± 11.59	78.03 ± 10.25	< 0.001
MAP (mmHg)	95.35 ± 11.04	104.71 ± 13.55	< 0.001
PP (mmHg)	45.52 ± 11.33	37.94 ± 7.77	< 0.001
baPWV (m/s)	7.65 ± 1.94	6.83 ± 1.10	0.022
AIx	21.49 ± 13.14	22.03 ± 15.60	0.88

ADPKD - autosomal dominant polycystic kidney disease, eGFR - estimated glomerular filtration rate, ACEi - angiotensin converting enzyme inhibitor, ARB - angiotensin receptor blocker, SBP - systolic blood pressure, DBP - diastolic blood pressure, MAP - mean arterial pressure, PP - pulse pressure, baPWV - brachial artery pulse wave velocity, AIx - augmentation index.

Table 2. Characteristics of patients with ADPKD.

	Control (n = 32)	CDK 1 (n = 15)	CKD 2 (n = 19)	CKD 3 (n = 22)	CKD 4 (n = 18)	p-values
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)	49.53 ± 7.32	30.62 ± 6.25	47.74 ± 9.39	59.09 ± 13.08	60.33 ± 14.11	< 0.001
Creatinine (mg/dL)	0.89 ± 0.13	0.82 ± 0.22	1.10 ± 0.31	1.63 ± 0.35	3 ± 0.79	< 0.001
eGFR (mL/min/1.73 m^2)	90.02 ± 20.39	109.04 ± 11.71	72.87 ± 13.43	41.83 ± 9.39	23.57 ± 20.36	< 0.001
Fibulin (ng/mL)	130.85 ± 176.96	230.67 ± 242.75	263.35 ± 227.16	321.61 ± 267.63	371.08 ± 290.17	< 0.001
baPWV (m/s)	6.83 ± 1.10	5.38 ± 0.58	7.22 ± 1.06	8.66 ± 1.73	8.67 ± 2.01	< 0.001
AIx	22.03 ± 15.6	20.31 ± 12.46	19.26 ± 12.84	21.32 ± 12.97	24.89 ± 14.49	0.8

eGFR - estimated glomerular filtration rate, baPWV - brachial artery pulse wave velocity, AIx - augmentation index.

significantly higher and eGFR was significantly lower in the patient group than in the healthy controls ($p < 0.001$). Hypertension was seen in 62.4% of patients, and 54% of them used angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB). Fibulin 1 was significantly higher in

Table 3. Correlation between levels of fibulin 1, baPWV, and clinical parameters of patients with ADPKD.

	Fibulin		baPWV	
	r	p	r	p
Age	0.127	0.197	0.881 **	0.001
eGFR (mL/min/1.73 m^2)	-0.364 **	0.001	-0.617 **	0.001
Creatinine	0.377 **	0.001	0.433 **	0.001
SBP (mmHg)	0.179	0.076	0.471 **	0.001
DBP (mmHg)	0.098	0.335	0.368 **	0.001
MAP (mmHg)	0.141	0.163	0.513 **	0.001
baPWV (m/s)	0.184	0.068		
AIx	-0.074	0.467	0.140	0.165

eGFR - estimated glomerular filtration rate, SBP - systolic blood pressure, DBP - diastolic blood pressure, MAP - mean arterial pressure, baPWV - brachial artery pulse wave velocity, AIx - augmentation index.

Table 4. Patient baseline characteristics stratified by plasma fibulin levels.

	Percentile group of fibulin				
	Group 1 (n = 36) 20.01 - 169.31 ng/mL		Group 2 (n = 37) 171.19 - 977.48 ng/mL		p-values
	Mean	Standard deviation	Mean	Standard deviation	
Age	49.69	16.02	52.24	15.69	0.490
Creatinine	1.49	0.86	1.89	1.00	0.066
eGFR (mL/min/1.73 m^2)	63.16	33.06	51.53	33.50	0.115
baPWV	7.51	1.97	7.82	1.95	0.421
SBP	127.34	16.81	129.06	13.70	0.443
DBP	83.20	11.26	85.70	12.12	0.373
MAP	104.51	15.08	105.00	12.18	0.606

eGFR - estimated glomerular filtration rate SBP - systolic blood pressure, DBP - diastolic blood pressure, MAP - mean arterial pressure, baPWV - brachial artery pulse wave velocity.

the patient group ($p < 0.001$). SBP, DBP, MAP, PP, and baPWV levels were also significantly higher in the patient group. AIx was not different between the patient and healthy group (Table 1).

The patients were classified into CKD stages based on the CKD-EPI equation: 20.2% were in CKD Stage 1, 25.6% were in CKD Stage 2, 29.7% were in CKD Stage 3, and 24.3% were in CKD Stage 4. Table 2 presents the main parameters for these CKD stage groups and the control group. One-way ANOVA revealed statistically significant differences between these groups for age ($p < 0.001$), creatinine ($p < 0.001$), and eGFR ($p < 0.001$). Significant differences were also detected for fibulin 1 and baPWV levels ($p = 0.010$) between the groups. AIx were not different between the groups. In the correlation analyses; a statistically significant positive correlation was found between fibulin 1 and creatinine ($r = 0.377$, $p = 0.001$). No significant correlation was found between the fibulin 1 levels and age, SBP, DBP, MAP, baPWV, and AIx. A statistically significant positive correlation was found between baPWV and age, creatinine, SBP, DBP, and MAP (Table 3). Based on their fibulin 1 levels, we divided the 74 patients into two groups: Group 1, 20.01 - 169.31 ng/mL (n = 36) and Group 2, 171.19 - 977.48 ng/mL (n = 37). No difference between the groups was found with regard to age, creatinine, eGFR, SBP, DBP, MAP, or baPWV levels (Table 4).

DISCUSSION

In the present study, we found elevated plasma concentrations of fibulin 1 in patients with ADPKD, and a statistically significant positive correlation was found between fibulin 1 and creatinine levels. These findings are in accordance with findings from a study showing that increased plasma fibulin 1 levels were associated with diabetes and impaired kidney function [10]. However, unlike in our study, that study found a correlation between age and fibulin levels. We could not find this correlation because the study population in the study by Scholze et al. had a higher average age (median age: 62.5 years) than our study group (median age: 49.0 years) [10].

Fibulin 1 is an extracellular matrix protein and is predominantly expressed in the great vessels during embryogenesis and has been found in human coronary artery atherosclerotic lesions; the protein is believed to participate in the pathophysiological processes leading to the progression and thrombotic complications of atherosclerosis [8]. Vascular dysfunction begins in the early stages, and hypertension is an early feature of ADPKD. In our study group, 62.4% of patients have hypertension. baPWV is widely used as an index of arterial stiffness in the general population because of its easy execution, good reproducibility, and good correlation with aortic PWV [13,14]. In our study, baPWV, AIx, SBP, DBP, and PP were measured in patients with ADPKD and healthy controls; baPWV and blood pressure measurements were significantly higher in patients than in controls. Impaired endothelium-dependent dilation and increased arterial stiffness measured by carotid femoral PWV are evident in the course of ADPKD in the presence of normal kidney function [15]. In a cohort of patients with ADPKD with preserved renal function, Gul et al. showed that the elasticity index was reduced in the small arteries [16].

AIx is a measure of the augmentation of the incident pulse wave due to reflection. It is calculated as the difference between the first and second derived aortic systolic peaks as a percentage of the pulse pressure. In a study conducted in normotensive patients with ADPKD, a significant increase in AIx was found [17]. Paapstel et al. showed an association between fibulin 1 and aortic AIx in male patients with peripheral arterial disease [18]. In our study, AIx was not different between the patient and control groups.

Cangemi et al. showed that in patients with diabetes, fibulin 1 was associated with pulse pressure, an indirect measure of arterial stiffness, and independently predicted total mortality during the 15 years of follow-up [9]. Laugesen et al. also showed a significant association between plasma fibulin levels and PWV in patients with type 2 diabetes [19]. In our study, factors affecting baPWV were age, eGFR, SBP, DBP, and MAP. No correlation was found between fibulin 1 and baPWV, and fibulin 1 and blood pressure parameters. Scholze et al. also found no correlation with fibulin concentrations and aortic PWV in patients with CKD. However, they found a positive correlation between AIx, central pulse pressure, and fibulin levels [10]. CKD duration, medications, gender distribution, and total kidney volume may be the factors affecting our results in our study population. Angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) was used in 54% of our patients with ADPKD. Aortic PWV was shown to be decreased in patients taking these medications [20-22]. We did not study the influence of the presence and absence of medication in our patients that could affect both the serum fibulin 1 levels and baPWV.

Our study had several limitations. It was a cross-sectional study, and the number of participants in the study was relatively small; larger studies are needed to confirm these results. For ethical reasons, patients could not be withdrawn from their medications to exclude any potential effects on hemodynamic parameters and biochemical marker levels. We did not measure kidney and cyst volumes, which may be associated with fibulin 1 levels. In addition, patients with end-stage kidney diseases undergoing renal replacement therapy were not included in this study.

CONCLUSION

To the best of our knowledge, this is the first study to demonstrate fibulin 1 levels in patients with ADPKD. Plasma concentrations of fibulin 1 increased in patients with ADPKD. ADPKD patients are usually monitored with serum creatinine levels and estimated glomerular filtration rate (eGFR); however, this gives limited information especially in the early stages of the disease. In recent years, total kidney volume is one of the methods that can be used to determine risk of progression to kidney failure in ADPKD patients, but it is expensive because magnetic resonance is used for the calculation. Fibulin levels may be an alternative parameter in the early stages of ADPKD. Arterial stiffness measured by baPWV increased in patients with ADPKD, but it was not related to fibulin 1 levels. The parameter with the greatest effect on fibulin 1 levels was eGFR.

Declaration of Interest:
The authors declare no conflict of interest.

References:

1. Davies F, Coles GA, Harper PS, Williams AJ, Evans C, Cochlin D. Polycystic kidney disease re-evaluated: a population-based study. Q J Med 1991;79(290):477-85 (PMID: 1946928).

2. Gabow PA. Autosomal dominant polycystic kidney disease. N Engl J Med 1993;329(5):332-42 (PMID: 8321262).

3. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet 2007;369:1287-301 (PMID: 17434405).

4. Ecder T, Schrier RW. Cardiovascular abnormalities in autosomal-dominant polycystic kidney disease. Nat Rev Nephrol 2009;5: 221-8 (PMID: 19322187).

5. Masoumi A, Elhassan E, Schrier RW. Interpretation of renal volume in autosomal dominant polycystic kidney disease and relevant clinical implications. Iran J Kidney Dis 2011;5:1-8 (PMID: 21189426).

6. Covic A, Gusbeth-Tatomir P, Goldsmith DJ. Arterial stiffness in renal patients: an update. AJKD 2005; 45(6): 965-77 (PMID: 15 957125).

7. Cangemi C, Hansen ML, Argraves WS, Rasmussen LM. Fibulins and their role in cardiovascular biology and disease. Adv Clin Chem 2014;67:245-65 (PMID: 25735864).

8. Argraves WS1, Tanaka A, Smith EP, et al. Fibulin-1 and fibrinogen in human atherosclerotic lesions. Histochem Cell Biol 2009; 132(5):559-65 (PMID: 19693531).

9. Cangemi C, Skov V, Poulsen MK, et al. Fibulin-1 is a marker for arterial extracellular matrix alterations in type 2 diabetes. Clin Chem 2011;57:1556-65 (PMID: 21926180).

10. Scholze A, Bladbjerg EM, Sidelmann JJ, et al. Plasma concentrations of extracellular matrix protein fibulin-1 are related to cardiovascular risk markers in chronic kidney disease and diabetes. Cardiovasc Diabetol 2013;12:6 (PMID: 23294625).

11. Neiman M, Hedberg JJ, Dönnes PR, et al. Plasma profiling reveals human fibulin-1 as candidate marker for renal impairment. J Proteom Res 2011;10:4925-34 (PMID: 21888404).

12. Joly D, Morel V, Hummel A, et al. Beta4 integrin and laminin 5 are aberrantly expressed in polycystic kidney disease: role in increased cell adhesion and migration. Am J Pathol 2003;63(5): 1791-800 (PMID: 14578180).

13. Yamashina A, Tomiyama H, Takeda K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359-64 (PMID: 12135313).

14. Tsuchikura S, Shoji T, Kimoto E, et al. Brachial-ankle pulse wave velocity as an index of central arterial stiffness. J Atheroscler Thromb 2010;17:658-65 (PMID: 20467192).

15. Nowak KL, Farmer H, Cadnapaphornchai MA, Gitomer B, Chonchol M. Vascular dysfunction in children and young adults with autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 2017;32(2):342-7 (PMID: 28186577).

16. Gul CB, Yildiz A, Ersoy A, et al. Correlation between arterial stiffness and inflammatory markers in autosomal dominant polycystic kidney disease patients with preserved renal function. Int Urol Nephrol 2015;47(7):1187-94 (PMID: 26047596).

17. Borresen ML, Wang D, Strandgaard S. Pulse wave reflection is amplified in normotensive patients with autosomal-dominant polycystic kidney disease and normal renal function. Am J Nephrol 2007;27(3):240-6 (PMID: 17389784).

18. Paapstel K, Zilmer M, Eha J, Tootsi K, Piir A, Kals J. Association Between Fibulin-1 and aortic augmentation index in male patients with peripheral arterial disease. Eur J Vasc Endovasc Surg 2016;51(1):76-82 (PMID: 26507503).

19. Laugesen E, Høyem P, Christiansen JS, et al. Plasma levels of the arterial wall protein fibulin-1 are associated with carotid-femoral pulse wave velocity: a cross-sectional study. Cardiovasc Diabetol 2013;12:107 (PMID: 23866070).

20. Mahmud A, Feely J. Reduction in arterial stiffness with angiotensin II antagonist is comparable with and additive to ACE inhibition. Am J Hypertens 2002;15:321-5 (PMID: 11991217).

21. Frimodt-Moller M, Kamper AL, Strandgaard S, Kreiner S, Nielsen AH. Beneficial effects on arterial stiffness and pulse-wave reflection of combined enalapril and candesartan in chronic kidney disease--a randomized trial. PLoS One 2012;7:e41757 (PMID: 22 860014).

22. Mitchell GF, Dunlap ME, Warnica W, et al. Long-term trandolapril treatment is associated with reduced aortic stiffness: the prevention of events with angiotensin-converting enzyme inhibition hemodynamic substudy. Hypertension 2007;49:1271-7 (PMID: 17452505).