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ORIGINAL ARTICLE

Association between urinary biomarkers and disease progression in adults with autosomal dominant polycystic kidney disease

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ABSTRACT

Background. Height-adjusted total kidney volume (htTKV) is considered as the best predictor of kidney function in patients with autosomal dominant polycystic kidney disease (ADPKD), but its limited predictive capacity stresses the need to find new biomarkers of ADPKD progression. The aim of this study was to investigate urinary biomarkers of ADPKD progression.

Methods. This observational study included ADPKD patients, and two comparator groups of ischaemic and non-ischaemic kidney injury: benign nephroangiosclerosis patients and non-ischaemic chronic kidney disease (CKD) patients. Proteinuria, htTKV and urinary levels of molecules are associated with ischaemia and/or tubular injury. The slope of estimated glomerular filtration rate (eGFR) was used as a dependent variable in univariate and multivariate models of kidney function decline.

Results. The study included 130 patients with ADPKD, 55 with nephroangiosclerosis and 40 with non-ischaemic CKD. All patients had increased urinary concentrations of biomarkers associated with tubular lesions (liver fatty acid-binding protein, kidney injury molecule-1, β2-microglobulin) and molecules overexpressed under ischaemic conditions [hypoxia-inducible factor-1α, vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1)]. These biomarkers correlated positively with htTKV and negatively with the eGFR slope. htTKV was the single best predictor of the eGFR slope variability in univariate analyses. However, a multivariate model including urinary levels of β2-microglobulin, MCP-1 and VEGF improved the capacity to predict the decline of eGFR in ADPKD patients compared with htTKV alone.

Conclusions. The urinary levels of molecules associated with either renal ischaemia (VEGF and MCP-1) or tubular damage (β2-microglobulin) are associated with renal function deterioration in ADPKD patients, and are, therefore, candidates as biomarkers of ADPKD progression.

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Keywords: autosomal dominant (ADPKD), biomarkers, disease progression, glomerular filtration rate, polycystic kidney, total kidney volume

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic kidney disease and is one of the leading hereditary causes of end-stage renal disease [1]. It is caused by the mutation of genes *PKD1* and *PKD2* and characterized by a proliferation of renal tubular cells leading to cyst formation and enlargement in the kidney and other organs [1]. As these cysts grow, they compress the renal parenchyma, causing tubulointerstitial damage and leading to ischaemic compromise and progressive renal failure in the long term in 50% of patients [2].

In ADPKD, the loss of renal function may remain undetected until the fourth decade of life, when renal cystic development is advanced and most nephrons have been destroyed [3]. Thus, it is important to find variables to identify patients who are at higher risk of disease progression, especially since there is evidence that treatment with tolvaptan—a vasopressin V2-receptor antagonist—may slow the deterioration of renal function [4]. PKD1 mutations (particularly truncated), hypertension, proteinuria and height-adjusted total kidney volume (htTKV) have been identified as primary predictors of ADPKD progression [5]. Of these, htTKV is currently considered the best predictor of the loss of renal function over time [6], although its predictive capacity is limited.

In addition to the aforementioned indicators, the concentration of molecules in urine, the expression of which is increased due to tubular injuries or under ischaemic conditions, may act as a predictor of renal function deterioration specifically associated with ADPKD and its mechanism of renal function impairment. These include molecules associated with tubular injuries, such as the kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), liver fatty acid-binding protein (L-FABP) or β2-microglobulin [7, 8]. Likewise, molecules overexpressed under ischaemic conditions, such as NGAL, hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1) can be candidates as indicators of cyst progression [9, 10]. Previous studies have investigated the potential prognostic value of several of these biomarkers without reaching conclusive results [11–15].

The limited capacity to predict of htTKV in ADPKD stresses the need to find new biomarkers—or combinations of biomarkers described—with good predictive value of ADPKD progression and risk of kidney injuries. The aim of this observational study was to analyse whether urinary levels of molecules related to tubular and/or ischaemic kidney damage have value as independent predictors of the slope of glomerular filtration rate (GFR) over time in adult patients with ADPKD.

MATERIALS AND METHODS

Study design and patients

This was an observational study carried out at two centres: Hospital Vall d'Hebron and Hospital Arnau de Vilanova, between January 2014 and May 2017. The study included three groups of patients: a main study group, which consisted of patients with an ultrasonographic diagnosis of ADPKD [16], and two comparator groups to obtain reference values for the excretion levels of the studied molecules in patients with ischaemic and non-ischaemic kidney injuries: nephroangiosclerosis and non-ischaemic chronic kidney disease (CKD) groups, respectively. The first comparator group consisted of patients with benign nephroangiosclerosis diagnosed by renal biopsy, and defined by the presence of characteristic vascular and glomerular findings [17, 18] and negative immunofluorescence studies, including C1q, IgG, IgA, IgM, C3, light chains and amyloid deposits. The other comparator group consisted of patients with biopsy-proven, non-ischaemic CKD, but with no vascular changes in kidney biopsies and no history of clinical vascular disease.

Patients were included if they were >18 years of age, gave their informed consent to participate in the research, and their GFR was >80 mL/min/1.73 m^2 at the time of study inclusion. ADPKD patients also had to have at least 10 years of follow-up and one GFR measurement per year after diagnosis. Exclusion criteria included pregnancy, urinary tract obstruction or infection, active infectious disease, previous history of neoplastic disease or active neoplasia, exposure to nephrotoxic drugs within 6 months prior to study inclusion, acute ischaemic vascular events in any vascular bed within 3 months before study entry and tolvaptan treatment. At the time of study entry, patients must not have been treated with cimetidine, cotrimoxazole or any other drug that could alter tubular creatinine secretion. ADPKD patients with asymmetrical or atypical cystic lesions or with a total kidney volume (TKV) that could not be measured by nuclear magnetic resonance were excluded from the study.

All patients signed an informed consent to participate in the study, which was approved by the local Ethics Committee.

Variables, measurements and endpoints

Demographic and clinical characteristics included age, gender, serum creatinine concentration, estimated GFR (eGFR) and the presence of comorbidities, such as diabetes mellitus and arterial hypertension. At study entry, spot, first morning urine samples were obtained from all patients to assess the presence of proteinuria and to analyse urinary levels of creatinine, MCP-1, VEGF, HIF-1α, NGAL, KIM-1, L-FABP and β2-microglobulin. ADPKD patients also underwent an MRI to measure their hTKV.

Creatinine measurements were performed using a compensated IDMS-traceable method (Hitachi Modular P-800 Roche Diagnostics, Berlin, Germany). Urinary MCP-1, VEGF, HIF-1α, NGAL, KIM-1 and L-FABP were quantified using ELISA kits from: R&D Systems (Minneapolis, MN, USA) for MCP-1 and NGAL, Abcam (Cambridge, UK) for HIF-1α and VEGF, CMIC (Tokyo, Japan) for L-FABP and Bio-Rad (Hercules, CA, USA) for KIM-1. All assays were performed in duplicate and calibrated with purified standards and reference samples from the manufacturers. Urinary β2-microglobulin was measured by immunoturbidimetry (Pacific Biomarkers, Seattle, WA, USA). In order to account for variations in urine flow rate, the concentrations of all molecules analysed were adjusted for urinary creatinine excretion. The reproducibility of urinary measurements was assessed by analysing the coefficients of variation of the studied molecules in a random sample of 10 ADPKD patients, after obtaining four consecutive first morning, spot urine samples within a 30-day period. GFR was estimated using the Chronic Kidney Disease

Table 1. Baseline characteristics of study patients

Variables	ADPKD (N = 130)	NAE (N = 55)	ni-CKD (N = 40)	Differences between groups (P-value)		
				ADPKD versus NAE	ADPKD versus ni-CKD	NAE versus ni-CKD
Demographic characteristics						
Age (years), mean (SD)	49 (21)	58 (23)	48 (34)	0.021	0.490	0.019
Male gender, *n* (%)	76 (58)	38 (69)	26 (65)	0.230	0.510	0.630
Clinical characteristics						
Comorbidities, *n* (%)						
Hypertension	88 (68)	53 (96)	26 (65)	0.000	0.750	0.000
Diabetes mellitus	9 (7)	10 (18)	2 (5)	0.021	0.620	0.560
Baseline eGFR (mL/min/1.73 m^2), mean (SD)	87 (13)	84 (34)	81 (32)	0.580	0.610	0.720
eGFR slope (mL/min/1.73 m^2/year), mean (SD)	−2.85 (1.72)	NA	NA			
htTKV (mL/m), mean (SD)	764 (390)	NA	NA			
Biomarkers urinary excretion levels						
Proteinuria (mg/g creatinine), median (IQR)	45 (15–143)	150 (56–250)	210 (110–200)	0.000	0.000	0.000
MCP-1 (ng/mg creatinine), median (IQR)	0.87 (0.4–1.3)	0.9 (0.5–2.2)	0.8 (0.5–1.9)	0.680	0.570	0.590
VEGF (ng/mg creatinine), mean (SD)	461 (225)	778 (139)	222 (96)	0.002	0.001	0.000
HIF-1α (ng/mg creatinine), mean (SD)	7.7 (3.8)	12.3 (6.4)	2.1 (1.8)	0.000	0.000	0.000
NGAL (μg/g creatinine), mean (SD)	37.0 (28.0)	35.9 (31.0)	38.2 (5.1)	0.720	0.660	0.550
KIM-1 (ng/g creatinine), mean (SD)	245 (166)	258 (231)	273 (135)	0.670	0.700	0.690
L-FABP (ng/mg creatinine), mean (SD)	55 (36)	56 (41)	48 (22)	0.490	0.530	0.560
B2MG (mg/g creatinine), mean (SD)	1.9 (0.8)	2.1 (1.2)	1.6 (1.3)	0.480	0.470	0.420

B2MG, β2-microglobulin [ref.* 0.18 (0.1)]; ni-CKD, non-ischaemic CKD; HIF-1α [ref.* 1.6 (0.5)]; KIM-1 [ref.* 130 (68)]; L-FABP [ref.* 16.8 (11.5)]; MCP-1 [ref.* 0.15 (0.09–0.5)]; NAE, nephroangiosclerosis [ref.* 14.2 (8.9)]. Asterisk is the reference value among healthy population in the study sites (internal record).

Epidemiology Collaboration (CKD-EPI) equation, as described by Levey *et al.* [19], and TKV was measured using the ellipsoid equation, as described by Irazabal *et al.* [20] and afterwards adjusted for height (htTKV).

The loss of renal function was defined as a decline in the eGFR slope during an observation period of 10 years. It was estimated using the least squares method, including at least 10 measurements of eGFR and assuming a linear progression model.

Statistics

Quantitative variables are described as mean and standard deviation (SD), or as median and interquartile range (IQR) for normal and non-normal distributions, respectively. Categorical variables are presented as frequency and percentage. Mean comparisons between groups were performed using an independent sample *t*-test, whereas proportions were compared using the Chi-square test. Correlation analyses between quantitative variables were performed using the Pearson correlation test. In order to identify predictors of the eGFR slope, a simple linear regression analysis was performed using the logarithm of the eGFR slope as the dependent variable, followed by a multiple regression model using the variables with a significant association in the univariate analysis. The threshold for statistical significance was established at a two-sided α value of 0.05. All statistical analyses were performed using the SPSS software (IBM SPSS Statistics for Windows, Version 20.0; IBM Corp., Armonk, NY, USA).

RESULTS

Characteristics of study patients

The study included 130 patients with ADPKD, 55 with nephroangiosclerosis and 40 with non-ischaemic CKD. Table 1 summarizes the main demographic and clinical characteristics of the three groups. Patients with nephroangiosclerosis were older and showed a higher frequency of hypertension compared with the ADPKD and non-ischaemic CKD groups. Proteinuria was significantly different between groups, being highest in non-ischaemic CKD patients and lowest in ADPKD ones. The highest levels of HIF-1α and VEGF were observed in patients with nephroangiosclerosis, followed by those with ADPKD and non-ischaemic CKD. No differences in the urinary concentrations of tubular injury markers MCP-1, NGAL, KIM-1, L-FABP and β2-microglobulin were observed between groups. Similarly, no inter-group differences were found for eGFR.

Coefficients of variation for the urinary concentrations of the studied molecules used to assess the reproducibility of urinary measurements were 11.0% for NGAL, 13.0% for KIM-1, 9.5% for L-FABP, 8.5% for MCP-1, 11.6% for HIF-1α, 9.3% for VEGF and 3.7% for β2-microglobulin.

Correlations of biomarkers with indices of disease progression

Table 2 shows the correlation matrix between the analysed variables in the three groups of patients. In all groups, eGFR correlated negatively with proteinuria, NGAL, KIM-1, L-FABP, β2-microglobulin, HIF-1α, VEGF and MCP-1, whereas positive correlations were observed between MCP-1, L-FABP, β2-microglobulin, HIF-1α and VEGF. In ADPKD patients, htTKV correlated positively with proteinuria, KIM-1, L-FABP, β2-microglobulin, HIF-1α, VEGF and MCP-1, but negatively with the eGFR slope, which also had a negative correlation with proteinuria, KIM-1, L-FABP, β2-microglobulin, HIF-1α, VEGF and MCP-1. Scatter plots of these correlations are provided in Supplementary data, Figure S1).

Table 2. Correlation matrix among variables in patients with ADPKD, NAE and non-ischaemic CKD

ADPKD										
Variable	eGFR	eGFR Slope	hTKV	NGAL	KIM-1	L-FABP	B2MG	Prot	HIF-1	VEGF
eGFR slope	−0.87									
hTKV	−0.46**	−0.46**								
NGAL	−0.31*	−0.09	0.09							
KIM-1	−0.33*	−0.29*	0.21**	0.26*						
L-FABP	−0.41*	−0.34*	0.32*	0.31*	0.37*					
B2MG	−0.47**	−0.45**	0.40**	0.24*	0.41**	0.29*				
Prot	−0.22*	−0.22*	0.35**	0.23*	0.44**	0.38**	0.34**			
HIF-1	−0.37**	−0.48**	0.38**	0.25*	0.31*	0.20*	0.48**	0.46**		
VEGF	−0.43**	−0.52**	0.43**	0.30*	0.13	0.28*	0.54**	0.55**	0.68**	
MCP-1	−0.48**	−0.64**	0.49**	0.26*	0.11	0.19*	0.46**	0.49**	0.46**	0.43**

NAE								
Variable	eGFR	NGAL	KIM-1	L-FABP	B2MG	Prot	HIF-1	VEGF
NGAL	−0.35*							
KIM-1	−0.33*	0.31*						
L-FABP	−0.37*	0.27*	0.30*					
B2MG	−0.52**	0.36*	0.39**	0.39*				
Prot	−0.54*	0.31*	0.46**	0.41**	0.43**			
HIF-1	−0.49**	0.23*	0.35*	0.30*	0.40**	0.51**		
VEGF	−0.61**	0.19	0.34*	0.32*	0.43**	0.48**	0.61**	
MCP-1	−0.68**	0.16	0.37*	0.21*	0.45**	0.44**	0.49**	0.58**

ni-CKD								
Variable	eGFR	NGAL	KIM-1	L-FABP	B2MG	Prot	HIF-1	VEGF
NGAL	−0.25*							
KIM-1	−0.29*	0.25*						
L-FABP	−0.28*	0.27*	0.30*					
B2MG	−0.32**	0.36*	0.39**	0.42*				
Prot	−0.31*	0.31*	0.46**	0.36**	0.27*			
HIF-1	−0.21*	0.16	0.35*	0.26*	0.35**	0.30**		
VEGF	−0.33*	0.19	0.29**	0.28*	0.39**	0.32**	0.48**	
MCP-1	−0.40**	0.13	0.20**	0.19*	0.44**	0.41**	0.31**	0.31**

B2MG, β2-microglobulin; ni-CKD, non-ischaemic CKD; NAE, nephroangiosclerosis.
*$P < 0.05$; **$P < 0.01$.

Regression analysis of eGFR slope as outcome parameter

The simple linear regression analysis showed that htTKV, proteinuria, KIM-1, L-FABP, β2-microglobulin, HIF-1α, VEGF and MCP-1 were significantly associated with the eGFR slope (Table 3). Among all these variables, htTKV was the single best predictor, explaining 31% of the eGFR slope variability. The multivariate model, including htTKV, β2-microglobulin, VEGF and MCP-1 as independent variables that significantly contributed to the model, explained 43% of the eGFR slope variability ($P < 0.001$) (Table 3).

Although the degree of association between the eGFR slope and tubular biomarkers KIM-1, β2-microglobulin and L-FABP was similar in the univariate analysis, when KIM-1 or L-FABP was introduced instead of β2-microglobulin, the regression model lost the capacity to explain the eGFR slope ($R^2 = 0.43$ for β2-microglobulin versus $R^2 = 0.33$ for KIM-1; $P = 0.024$ and $R^2 = 0.43$ for β2-microglobulin versus $R^2 = 0.35$ for L-FABP; $P = 0.029$). Likewise, when VEGF was replaced by HIF-1α, the capacity to explain the eGFR slope of the model was significantly reduced ($R^2 = 0.43$ for VEGF versus $R^2 = 0.34$ for HIF-1α; $P = 0.042$).

DISCUSSION

In this observational study, we found that ADPKD patients have increased urinary concentrations of biomarkers associated with tubular lesions (L-FABP, KIM-1 and β2-microglobulin) and renal ischaemia (HIF-1α, VEGF and MCP-1), and that these biomarkers correlate with the current standard measure to monitor htTKV in ADPKD progression. Compared with htTKV alone, a multivariate model including the levels of β2-microglobulin, MCP-1 and VEGF in urine improved the capacity to predict the decline of eGFR in these patients.

Consistently with previous studies investigating biomarkers for ADPKD progression [10–15, 21], ADPKD patients in our study had higher urine levels of molecules associated with tubular injuries such as KIM-1, L-FABP, NGAL and β2 microglobulin than those typically found in healthy people. However, the mean level of these molecules was similar to that found in patients with other types of renal injury (i.e. nephroangiosclerosis and non-ischaemic CKD), indicating little specificity for ADPKD. Conversely, the levels of HIF-1α and VEGF were significantly higher in patients with ischaemic injury (i.e. nephroangiosclerosis and ADPKD) than in patients with non-ischaemic CKD without suspicion of renal ischaemia. Furthermore, the remarkably

Table 3. Predictors of the eGFR slope in univariated and multivariated regression models

Variables	B	t	P	R^2
Univariated				
htTKV	−0.65	−6.2	0.000	0.31
KIM-1	−0.26	−2.5	0.015	0.12
L-FABP	−0.22	−2.6	0.026	0.23
Proteinuria	−0.30	−3.3	0.009	0.19
B2MG	−0.34	−3.8	0.005	0.21
HIF-1α	−0.41	−4.1	0.000	0.23
VEGF	−0.44	−4.9	0.000	0.24
MCP-1	−0.47	−3.9	0.007	0.22
Multivariated				
htTKV	−0.52	−5.0	0.000	0.43
VEGF	−0.19	−3.3	0.024	
MCP-1	−0.15	−3.1	0.029	
B2MG	−0.11	−2.1	0.041	

B2MG, β2-microglobulin.

higher levels of proteinuria in non-ischaemic CKD patients indicate that the increased level of these molecules cannot be attributed to the overall increase in urine excretion of proteins. This finding suggests that the pressure of the growing cysts induces a hypoxia environment, which stimulates the expression and release of HIFs, including HIF-1α and the pro-angiogenic gene *VEGF* [22, 23], which in turn may upregulate the expression of MCP-1 [24], a potent chemotactic factor for monocytes that plays an important role in inflammatory processes [25]. To our knowledge, this is the first work reporting an increase of HIF-1α and VEGF in ADPKD patients. Previous studies have also described higher urinary concentrations for MCP-1 in these patients [10, 11, 14]. However, unlike HIF-1α and VEGF, MCP-1 levels did not differ between groups in our study. Of note, although MCP-1 has been associated with hypoxia under *in vitro* conditions [26], this molecule is also released in response to other stimuli [25, 27], which may have contributed to the different behaviour of MCP-1 compared with HIF-1α and VEGF.

Irrespective of the differences in the urinary levels of these molecules between groups, the levels of the three molecules associated with renal hypoxia significantly correlated with each other in all groups of patients, which is consistent with the pathway involving HIF-1α, VEGF and MCP-1 suggested earlier. In patients with ADPKD, biomarkers related with tubular injuries (KIM-1, β2-microglobulin and L-FABP) and renal ischaemia (HIF-1α, VEGF and MCP-1) showed a weak-to-moderate correlation with the current standard of htTKV in ADPKD progression. Whereas these associations have been described for tubular biomarkers, such as KIM-1 and NGAL [11, 13], and for MCP-1 [10, 11, 28], the association of HIF-1α and VEGF with htTKV has not been reported previously.

When assessing the capacity of the investigated molecules to explain the eGFR slope in a univariate model, htTKV remained the biomarker accounting for the greatest percentage (31%) of variability in the eGFR slope. However, the introduction of β2-microglobulin, VEGF and MCP-1 levels improved the strength of the model, increasing the percentage of the eGFR slope variability nearly 10 points. Other models, including that replacing VEGF by HIF-1α, had lower capacity to predict change in the eGFR slope. Although the mechanism of hypoxia in ADPKD patients was out of the scope of our research, this finding suggests that HIF-1α levels may remain rather unchanged during the progression of ADPKD and, therefore, be less sensitive to changes in the eGFR slope.

This study was strengthened by the inclusion of two comparison groups, which allowed us to discern a scenario of general injuries and of ischaemic injuries. However, our results have some limitations that need to be considered. First, GFR was estimated retrospectively using the CKD-EPI equation from data gathered from historical records. Nevertheless, whereas this limitation introduces a source of variability in the measurement of the disease progression, it rather affects all patients equally and, therefore, it is unlikely to result in a non-proportional bias. Secondly, due to the observational design, candidate molecules could not be measured before the deterioration of renal function. Hence, although in multivariate analyses, we found evidence of an independent association between these variables and the slope of eGFR, it was not possible to analyse whether the levels of these urinary biomarkers are useful to predict future deterioration of the renal function before observing a decline in the eGFR slope. Another consequence of the observational design was the unbalanced age of the groups at baseline, as recruitment was constrained by patients admitted in routine practice. Thirdly, all measurements were made in spot urine samples, and its consistency with total excretion in 24-h urine was not analysed; therefore, results could differ depending on the urine sample used. Although both analytical and within-subject variability in repeated measurements was low, biological variability of the urinary excretion of the different molecules studied is not known, being of crucial importance before considering its potential applicability in clinical practice. Finally, no genetic data of study participants were available, which precluded introducing the influence of the type of mutation on the eGFR slope in the multivariate models.

In summary, our results provide clinical evidence indicating that, in addition to htTKV, the urinary levels of molecules associated with either renal ischaemia (VEGF and MCP-1) or tubular damage (β2-microglobulin) are associated with renal function deterioration in patients with ADPKD, and may therefore be used as biomarkers of kidney injuries in ADPKD patients with mild reduction of GFR. Future studies investigating the performance of these biomarkers prospectively are guaranteed.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

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AUTHORS' CONTRIBUTIONS

A.S.-M., I.A. and M.Martin have made a substantial contribution to the conception and design of the study, as well as

the collection of data, statistical analysis and drafting the work. L.C.-C. and A.G.-C. performed the collection and biochemical determinations of the biological samples. S.R. oversaw all MRI and kidney volume measurements. M.V., B.C., M.Molina and E.J. were in charge of the recruitment, selection and health control of the patients as well as the recollection of biologic samples. All co-authors have revised all manuscript drafts critically and have approved the final version of the manuscript. All co-authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work were appropriately addressed and resolved.

CONFLICT OF INTEREST STATEMENT

None declared.

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