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NEPHROLOGY - ORIGINAL PAPER

Urinary citrate as a marker of renal function in patients with autosomal dominant polycystic kidney disease

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Abstract

Introduction Autosomal dominant polycystic kidney disease (ADPKD) is frequent to find low urinary citrate levels. Recently, it has been suggested that urinary citrate could be a marker of covert metabolic acidosis in chronic kidney disease.

Objective Our aim was to analyze relationship between urinary citrate levels, renal function, and serum bicarbonate in ADPKD patients.

Methods We determined citrate in 24-h collected urine from ADPKD patients and correlated with glomerular filtration rate (CKD-EPI equation) and serum bicarbonate concentration.

Results We included 120 patients, 60% men, eGFR was 71 ± 32 mL/min/1.73 m^2. Urinary citrate/creatinine ratio was 195 ± 152 mg/gCr (range 1.2–689) with levels significantly higher in females. Urinary citrate lower than 300 mg/gCr was present in 75% of patients and when considering chronic kidney stages (CKD), we observed reduced levels in 48.8% in CKD1 stage, in 79.4% in CKD2 stage, in 96.2% in CKD3 stage, and in 94.7% of patients in CKD4 stage. Urinary citrate was correlated with serum creatinine ($r = -0.61$, $p < 0.001$) and eGFR ($r = 0.55$, $p < 0.001$) in both gender. We did not find any correlation with serum bicarbonate. Using a general linear modeling analysis, we found as predictors of urinary citrate/creatinine ratio to glomerular filtration rate, gender, and age. Lower levels of urinary citrate were accompanied by a decline in urinary osmolality and in renal excretion of calcium and uric acid. In a subgroup of patients, we measured total kidney volume and we found an inverse correlation with urinary citrate levels that disappeared when it was corrected with glomerular filtration rate.

Conclusions Urinary citrate is very frequently reduced in ADPKD patients being present from very early CKD stages. Their levels in urine are inversely correlated with glomerular filtration rate and it is not related with serum bicarbonate concentration. We think that it would be interesting to study urinary citrate as a marker of chronic kidney disease in ADPKD patients.

Keywords Polycystic kidney disease · Citrate · Acidosis · Chronic kidney disease

Introduction

Citrate is a tricarboxylic acid with a central role in Krebs cycle that is used by kidneys as an important metabolic source. Only 1% comes from diet and urine appearance is the result of glomerular filtration and subsequent resorption in proximal tubule mediated by specific transporters [1, 2]. Urinary citrate excretion is influenced by urinary acidification and urinary calcium concentration [3, 4], while potassium seems to influence citrate concentration through degree of intracellular acidification [1].

Hypocitraturia is observed in 54–67% of patients with autosomal dominant polycystic kidney disease (ADPKD) [5, 6]. This finding is often accompanied by hyperoxaluria in 18–19.4%, hypercalciuria in 11%, hypomagnesiuria in 29%, and hyperuricuria in 15%. The study of all these factors have been oriented toward the development of kidney stones that are very frequent in ADPKD [5, 7, 8]. This high prevalence of hypocitraturia has been attributed to a possible deficit in ammonium generation secondary to damage of interstitium

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and tubular atrophy caused by the growing of cysts, resulting in a deficit of urinary buffering that might favor reabsorption of urinary citrate [8, 9]. However, in spite of this buffering deficit, urinary pH remains acidic in the majority of patients [8, 10].

In a recent publication, Goraya et al. [11] suggest that urinary citrate could be used as a marker of acid retention in patients without obvious metabolic acidosis with normal serum bicarbonate levels. Authors analyzed in patients with arterial hypertension with chronic kidney disease (CKD) in stages 1 and 2, the response to an alkali-rich diet based in fruits and vegetables, and observed a reduction in acid retention that was correlated with an increase of urinary citrate [11]. In addition, authors showed in a randomized study that bicarbonate administration in CKD2 patients was able to preserve renal function, showing that urinary citrate reflected a better control of acid overload [12]. Acid retention increases with progression of renal failure [13] and, although its measurement is laborious, urinary citrate determinations could be a very simple way to estimate such acid retention [14].

In a previous work, we studied urinary citrate levels in patients with CKD secondary to diverse etiologies and we found that it was common to observe a reduction in their levels in urine when renal function is impaired [15]. This reduction was present not only in ADPKD but also in other etiologies which indicates that this alteration is typical of the progression of renal disease. Therefore, it seems necessary to analyze citrate levels in urine in a systematic way in patients with ADPKD, showing its relationship with deterioration of glomerular filtration and presenting data about what other modifications are present when urinary citrate is reduced, what constitutes the aims of the present work.

Methods

This study was conducted in patients with ADPKD regularly visited in an outpatient renal clinic, excluding patients with glomerular filtration rate (GFR) lower than 15 mL/min/1.73 m^2 (CKD5 stage). Diagnosis of ADPKD was based on radiological findings (ultrasound and/or computed tomography) and a family history of polycystic kidney disease (PKD) [16, 17]. In these patients, determinations of urinary citrate, calcium, and uric acid in 24-h urine are routinely performed, regardless if they presented with renal lithiasis or not. All patients undergoing follow-up in our renal clinic have signed an informed consent allowing to use their analytical and demographic data anonymously in research studies. This work is a retrospective study that collected blood and urine determinations available in the records of our central laboratory. Patients did not follow any specific diet before analytical determinations in addition to dietary recommendations usually made for their CKD stage. Patients collected 24-h urine according to usual procedure without adding any chemical to the urine sample.

Urinary citrate was determined by an enzymatic method using ultraviolet spectrophotometry with a commercial kit manufactured by Boehringer Mannheim. In a first step, citrate present in sample is transformed into oxaloacetate and acetate by means of citrate lyase [18]. Then, oxaloacetate is transformed into malate through malate dehydrogenase which, in turn, generates lactate by action of lactate dehydrogenase. In these latter reactions, coenzyme $NADH/NAD^+$ is involved and its concentration can be determined by absorptiometry at 340 nm. Increase in absorbance is proportional to citrate concentration [18]. Urinary values > 300 mg/g creatinine were considered normal in both men and women.

Creatinine was measured by Jaffé's modified method without traceability for IDMS. Calcium was determined by photometry using the *O*-cresolphthalein complexone reagent and uric acid using uricase method. All these biochemical parameters were determined in a Roche Cobas c702 autoanalyzer. Glomerular filtration rate was estimated using the CKD-EPI equation (eGFR) [19]. Urinary osmolality was determined using an Osmomat 030 cryoscopic osmometer (sensitivity of 1 mOsm/Kg, coefficient of variation < ±0.5%).

Statistical analysis was performed with SPSS r22 statistical package. Data are expressed as means ± standard deviations or as frequencies as it is required. Normality of distribution of variables was verified using Kolmogorov–Smirnov test. When they did not have a normal distribution, variables were log-transformed for their analysis. For comparison of two unpaired groups, we used Mann–Whitney test and for more unpaired groups the Kruskal–Wallis test. Association between two categorical variables was studied by Pearson χ^2 test. We used Spearman coefficients to study correlations between quantitative variables. To build predictor models adjusting by covariates of urinary citrate levels, we used general linear modeling (GLM) analysis. We consider significant values when $p < 0.05$.

This paper was redacted in according to the recommendations of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [20].

Results

We studied 120 patients, 72 (60%) men, aged 47 ± 16 years. Mean serum creatinine was 1.34 ± 0.69 mg/dL, median 1.10 mg/dL, and range 0.60–3.77 mg/dL. eGFR was 71 ± 32 mL/min/1.73 m^2, median 69, and range 15–139 mL/min/1.73 m^2. 41 (34.2%) patients were in CKD1 stage, 34 (28.3%) in CKD2 stage, 26 (21.7%) in CKD3 stage, and 19 (15.8%) in CKD4 stage.

Mean urinary concentration of citrate was 151 ± 149 mg/L, median 103 mg/L, and with a range of 0.5–724 mg/L. The daily excretion of citrate in urine was 279 ± 226 mg/day, median 234 mg/day, and ranged from 1 to 1013 mg/day. Urinary citrate/creatinine ratio was 195 ± 152 mg/gCr, median 163 mg/gCr, and fluctuated from 1.2 to 689 mg/gCr. All results showed a very asymmetric distribution with significant differences with respect to a normal distribution using Kolmogorov–Smirnov test, not being corrected at all after its logarithmic transformation.

Urinary citrate/creatinine ratio was significantly higher in women than in men (249 ± 175 vs 159 ± 123 mg/gCr, respectively, $p < 0.003$) but not when values are presented as concentrations (women 171 ± 155, men 139 ± 145 mg/L, $p = 0.257$) or as daily urinary excretion (women 304 ± 243 mg/day, men 263 ± 215 mg/day, $p = 0.330$). We also found lower urinary creatinine concentrations in women than in men (61.4 ± 26.4 mg/dL vs 82.7 ± 36.1 mg/dL respectively, $p < 0.001$) and a lower daily urinary creatinine excretion (women 1169 ± 331 mg/day, men 1697 ± 567 mg/day, $p < 0.001$). This lower excretion of creatinine in women may explain in part this difference observed in citrate/creatinine ratio, which is the most frequent way of presenting the results of this substance in the literature.

Similarly, the daily excretion of uric acid was lower in women than in men (507 ± 169 mg/day vs 611 ± 346 mg/day, respectively, $p = 0.033$), but with higher uric acid/creatinine ratio (women 431 ± 92 mg/gCr, men 357 ± 149 mg/gCr, $p = 0.001$). Daily excretion of calcium and calcium/creatinine ratio were not different according to gender.

In Table 1, we show serum and urinary parameters according to CKD stages. Serum uric acid levels increased significantly with CKD stage. Bicarbonate was only lower in CKD4 stage. Urinary output rose with CKD stage but not significantly, while osmolality decreased specially in CKD 4 stage. Urinary citrate decreased from early CKD stages in all ways of presentation. Urinary citrate levels lower than 300 mg/gCr were present in 75% of patients, and when considering CKD stages, we observed reduced levels in 48.8% in CKD1 stage, in 79.4% in CKD2 stage, in 96.2% in CKD3 stage and in 94.7% of patients in CKD4 stage. Urinary excretion of uric acid and calcium also progressively decreased at each CKD stage. Albuminuria and proteinuria rose to become only significant in CKD4 stage.

Urinary citrate/creatinine ratio was negatively correlated with serum creatinine ($r = -0.61$, $p < 0.001$) following an inverse relationship (Fig. 1), and positively correlated with eGFR ($r = 0.55$, $p < 0.001$). Citrate

Table 1 Comparative of age, gender distribution, estimated glomerular filtration rate (eGFR) using CKD-EPI equation and levels of urinary and serum parameters evaluated in the study according to chronic kidney disease (CKD) stages

	CKD1 ($n = 41$)	CKD2 ($n = 34$)	CKD3 ($n = 26$)	CKD4 ($n = 19$)	p*
Age (years)	35 ± 12	44 ± 12	60 ± 11	59 ± 14	< 0.001
Gender (%Male/%Female)	58.5/41.5	55.9/44.1	76.9/23.1	47.4/52.6	0.200 &
Serum					
Urea (mg/dL)	35 ± 7	42 ± 11	58 ± 10	88 ± 28	< 0.001
Creatinine (mg/dL)	0.82 ± 0.13	1.10 ± 0.19	1.58 ± 0.31	2.60 ± 0.69	< 0.001
Uric acid (mg/dL)	4.9 ± 1.4	5.3 ± 1.1	6,7 ± 1,7	6,8 ± 1,3	< 0.001
Calcium (mg/dL)	9.5 ± 0.4	9.5 ± 0.5	9.7 ± 0.4	9.6 ± 0.3	0.647
Bicarbonate (mEq/L)	25.3 ± 2.9	25.4 ± 2.9	24.8 ± 3.0	22.9 ± 2.5	0.014
eGFR-CKD-EPI (mL/min/1.73 m^2)	108 ± 11	74 ± 10	46 ± 9	24 ± 5	< 0.001
Urine					
Urine output (mL/day)	2017 ± 805	2336 ± 1024	2216 ± 623	2358 ± 698	0.084
Osmolality (mOsm/Kg)	584 ± 206	440 ± 168	433 ± 84	337 ± 92	< 0.001
Citrate (mg/L)	238 ± 171	162 ± 136	81 ± 77	42 ± 56	< 0.001
Citrate (mg/g creatinine)	289 ± 171	211 ± 119	111 ± 76	77 ± 91	< 0.001
Citrate excretion (mg/day)	413 ± 247	313 ± 195	166 ± 119	87 ± 108	< 0.001
Uric acid (mg/g creatinine)	468 ± 114	386 ± 108	352 ± 117	258 ± 121	< 0.001
Uric acid excretion (mg/day)	739 ± 352	575 ± 200	491 ± 161	283 ± 119	< 0.001
Calcium (mg/g creatinine)	125 ± 62	96 ± 52	56 ± 41	31 ± 29	< 0.001
Calcium excretion (mg/day)	199 ± 141	144 ± 71	78 ± 66	33 ± 29	< 0.001
Albuminuria (mg/g creatinine)	44 ± 90	30 ± 31	110 ± 227	347 ± 902	< 0.001
Proteinuria (mg/g creatinine)	168 ± 248	116 ± 60	237 ± 314	660 ± 1320	0.002

*Kruskal–Wallis test

&Pearson χ^2 test

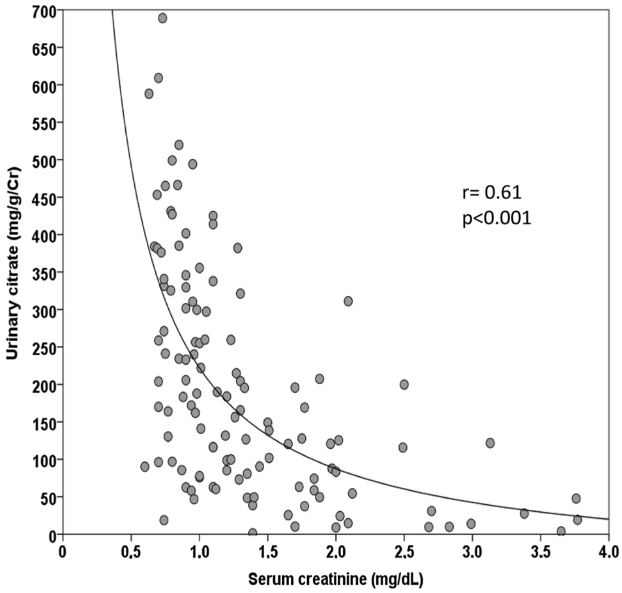

Fig. 1 Distribution of urinary citrate/creatinine ratio versus serum creatinine concentration. There was a hyperbolic relationship between both variables without differences between females and males

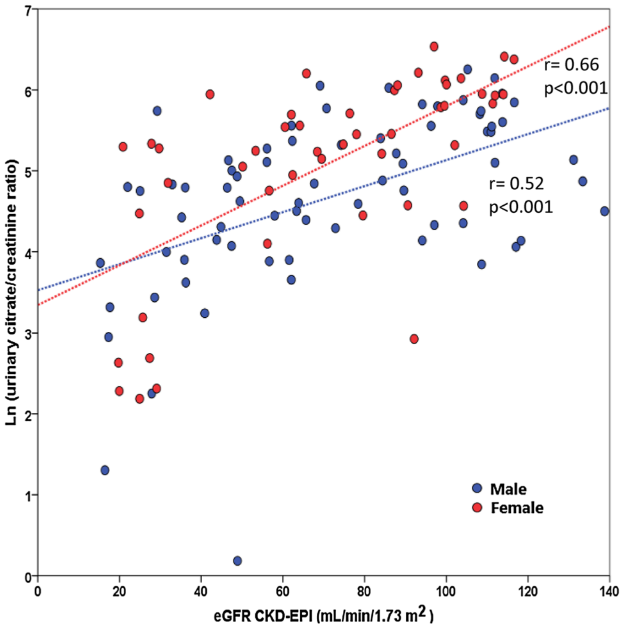

Fig. 2 Urinary citrate/creatinine ratio was significantly correlated with glomerular filtration rate estimated with CKD-EPI equation (eGFR). Values of urinary citrate in women were slightly higher than in males for the same eGFR, with significant correlations separately

concentration in urine and daily excretion also correlated with serum creatinine (both with $r = -0.60$ and $p < 0.001$) and with eGFR (both with $r = 0.62$ and $p < 0.001$). When we segmented patients by gender, we also found significant correlations with eGFR (males $r = 0.52$, females $r = 0.66$, both $p < 0.001$) and different regression lines for men and for women (Fig. 2). Linear regression equation showed an increase in urinary citrate/creatinine by 24.67 mg/gCr per 10 mL/min/1.73 m^2 of eGFR and 92.3 mg/gCr for women compared to men. Age also correlated with citrate in all ways of presentation with Spearman coefficient that ranged from $r = -0.23$ to -0.37 ($p < 0.001$ for all).

We did not find any correlation between urinary citrate with serum bicarbonate levels (Fig. 3). Urinary citrate concentration positively correlated with urinary uric acid ($r = 0.68$, $p < 0.001$) and urinary calcium ($r = 0.58$, $p < 0.001$) concentrations, with urinary osmolality ($r = 0.53$, $p < 0.001$) and mildly with albuminuria ($r = -0.28$, $p = 0.002$) and proteinuria ($r = -0.21$, $p = 0.025$).

Using a general linear modeling (GLM), we searched for predictors for urinary citrate levels selecting as independent variables to gender, age, and serum creatinine (or eGFR). When we tried to predict citrate as concentrations or as daily excretion, only serum creatinine or eGFR were significant predictors. However, when we used the urinary citrate/creatinine ratio, the significant variables chosen were gender (female 87.12 mg/gCr over male), age (2.12 mg/gCr per year) and eGFR (31.9 per 10 mL/min/1.73 m^2) with adjusted $r^2 = 0.37$.

We classified patients in three groups according to urinary citrate tertiles: < 96, 96–238 and > 238 mg/gCr. Figure 4 shows patient proportion in each tertile according to CKD stage. In CKD1 stage, 17.1% of patients exhibited very low levels, belonging to the lower tertile, as well as 18.9%

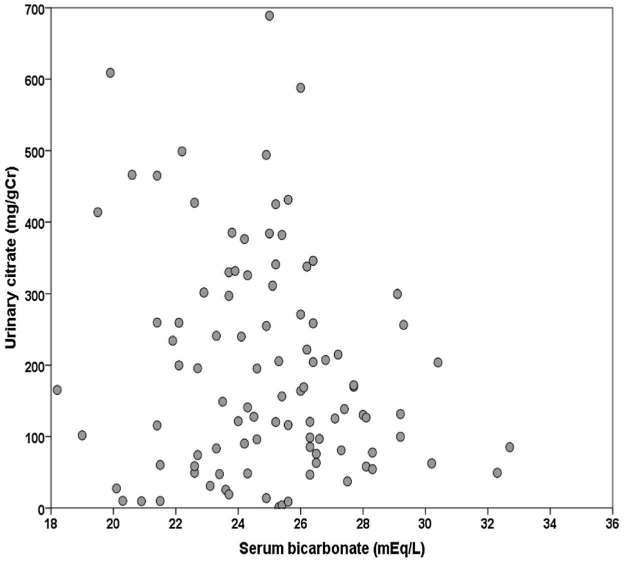

Fig. 3 We did not observe correlation between urinary citrate levels and serum bicarbonate concentration

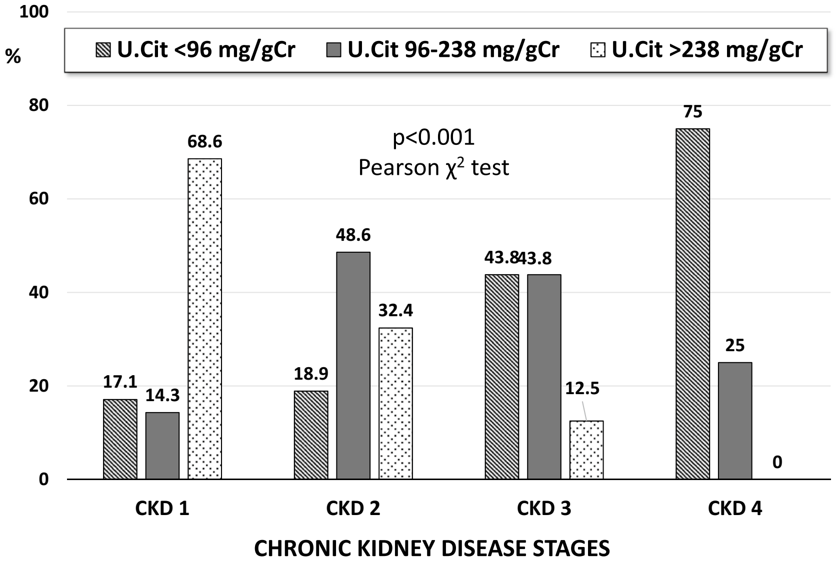

Fig. 4 Distribution of patients according to tertiles of urinary citrate and stages of chronic kidney disease (CKD). As the stage of CKD progressed, proportion of patients belonging to the upper tertile is reduced and patients in the lower tertile increased

in CKD2 stage. Only 12.5% maintained levels in the upper tertile in CKD3 stage and none in CKD4 stage.

Table 2 shows some data stratified according to urinary citrate tertiles. We did not observe any difference in serum bicarbonate levels, while a progressive increase of serum uric acid was present accompanying to a significant reduction in eGFR in the lower tertile. Urinary osmolality and urinary uric acid and calcium/creatinine ratios were also significantly lower in patients belonging to the lower tertile, while albuminuria and proteinuria significantly rose their levels only in this tertile.

In 82 patients, we used computed tomography or magnetic resonance imaging to estimate total kidney volume adjusted by height (TKV/ht) using the ellipsoid formula [21]. They were 46 ± 13 years old and 46 (56.1%) men. eGFR was 69 ± 30 ml/min/1.73 m^2 and TKV/ht was 1128 ± 1131 mL/m (median 829). Urinary citrate was 188 ± 140 mg/gCr, with a median of 153 and a range from 3.7 to 588 mg/gCr. TKV/ht was inversely correlated with urinary citrate as it is shown in Fig. 5 ($r = -0.41$, $p < 0.001$). Using GLM, we found that gender and eGFR were the main predictors of urinary citrate ($r^2 = 0.46$, $p < 0.001$), losing TKV/ht significance. Urinary uric acid and calcium did not show any significant relationship either with TKV/ht when we adjusted by eGFR.

Table 2 Renal function, serum and urinary parameters determined in patients with autosomal polycystic kidney disease (ADPKD) according to tertiles of urinary citrate/creatinine ratio

	Urinary citrate < 96 mg/gCr	Urinary citrate 96–238 mg/gCr	Urinary citrate > 238 mg/gCr	p*
Urea (mg/dL)	64 ± 29	48 ± 18	38 ± 11	< 0.001
Creatinine (mg/dL)	1.81 ± 0.85	1.32 ± 0.54	0.92 ± 0.26	< 0.001
Uric acid (mg/dL)	6.4 ± 1.4	5.9 ± 1.6	4.8 ± 1.3	< 0.001
Calcium (mg/dL)	9.6 ± 0.4	9.7 ± 0.4	9.5 ± 0.4	0.207
Bicarbonate (mEq/L)	24.7 ± 3.6	25.4 ± 2.7	24.2 ± 2.3	0.072
eGFR CKD-EPI (ml/min/1.73m^2)	54 ± 33	66 ± 28	93 ± 22	< 0.001
Urine				
Urine output (mL/day)	2262 ± 545	2339 ± 903	2011 ± 951	0.016
Osmolality (mOsm/Kg)	426 ± 166	433 ± 134	554 ± 216	0.008
Citrate (mg/g creatinine)	47.7 ± 28.2	158.4 ± 41.3	376 ± 105.8	< 0.001
Uric acid (mg/g creatinine)	328 ± 142	366 ± 116	465 ± 105	< 0.001
Calcium (mg/g creatinine)	55.3 ± 44.0	90.1 ± 54.1	116.5 ± 67.4	< 0.001
Albuminuria (mg/g creatinine)	222 ± 659	38 ± 48	51 ± 95	0.128
Proteinuria (mg/g creatinine)	411 ± 969	139 ± 118	195 ± 262	0.110

*Kruskal–Wallis test

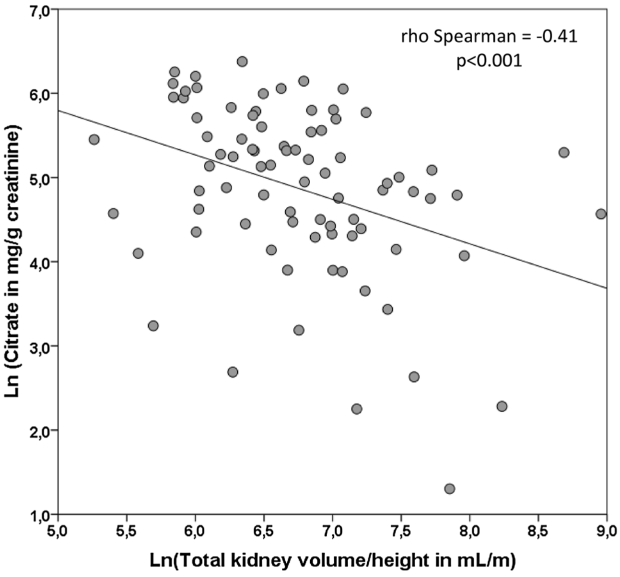

Fig. 5 Relationship between urinary citrate with total kidney volume adjusted for height (TKV/h) measured with computed tomography or resonance magnetic imaging in patients with autosomal dominant polycystic kidney disease. Both variables were significantly correlated (both variables are presented transformed with natural logarithm). There were no differences when considering gender separately

Discussion

The main result of the present study is that we can observe a progressive reduction of urinary citrate concentration in ADPKD patients when GFR is impaired, that in some cases may be present very early, when it is still quite conserved. For example, we have found that 48.8% of patients in CKD1 stage and 79.4% in CKD2 stage exhibited reduced levels. This observation is the first time that is mentioned in PKD, given that in the previous works, this prevalence is globally evaluated and not considering the degree of GFR.

From a mechanistic point of view, citrate excretion in urine depends on three main factors: the glomerular filtration rate, the urinary pH, and calcium concentration in urine.

Urinary citrate comes exclusively from glomerular filtration, but is reabsorbed by 65–90% in proximal tubule mediated by a dicarboxylic acid transporter, a mechanism that is shared with ketoglutarate or succinate [1]. In urine, citrate is presented dissociated as citrate^{3-} which will be transformed into citrate^{2-} in presence of a urinary acid pH, without ligating with calcium or magnesium, and binding to NaDC-1 transporter for its reabsorption [3]. Inside the tubular cell, it is transported inside mitochondria where it is incorporated into oxidative metabolism in tricarboxylic acids cycle, being an important energy source for kidney [1–3]. Citrate consumption by tubular cells depends on intracellular pH, so that, in presence of intracellular acidosis, it accumulates slowing its entry into cells [3].

Progressive GFR reduction might explain a decrease in filtered load of urinary citrate [1, 22, 23] and its reduction in urine, since the maximum capacity of tubular re-uptake is well above the degree of reduction of GFR. Therefore, a basic explanation of why urinary citrate is so reduced in ADPKD patients would simply be the GFR reduction. However, the decline in urinary citrate is much more intense than the degree of deterioration of the GFR which suggests that other mechanisms might be involved.

Acidosis and metabolic alkalosis clearly influence the urinary citrate [1–3]. Metabolic acidosis causes an acidic urinary pH in proximal tubule, which facilitates the shift of citrate^{3-} to citrate^{2-} increasing its reabsorption by a dicarboxylic acid receptor, with consequent reduction in urinary levels. On the contrary, metabolic alkalosis causes a rise in urinary pH which facilitates the shift of citrate^{2-} to citrate^{3-}, reducing its reabsorption and increasing its urinary excretion. Urinary pH significantly influences citrate elimination. A reduction in urinary citrate can be observed in systemic acidosis caused by carbonic anhydrase inhibitors such as acetazolamide [1, 3] or topiramate [24]. An increase of urinary citrate is observed with potassium bicarbonate or potassium citrate administration [25]. Therefore, the presence of metabolic acidosis can be a very interesting mechanism that may explain the low urinary levels found in ADPKD.

Urinary acidification in PKD is altered from very early stages, although urinary pH is not increased [8, 10]. When PKD patients are subjected to an acid load with ammonium chloride, the ability to remove this excess of acid is clearly reduced due to a reduced generation of ammonium in renal medulla [8, 10]. It is possible that this reduced production of ammonium is accompanied by an acidic tubular pH that would favor citrate reabsorption in greater amounts than normal, even though they do not present a severe reduction of GFR. It was postulated that this phenomenon is related with the destruction of the renal medulla by the growth of cysts [10] and that it is the explanation for the frequent hypocitraturia detected in PKD patients, which can reach to 54–67% in some series [5, 8, 24]. However, hypocitraturia can be present very early in PKD with renal function still conserved as we have observed in some patients in CKD1 and CKD2 stages. In these patients, it is difficult to think that cysts have damaged so much the medullar structure as to explain such a reduction in renal ammoniogenesis. Moreover, this hypocitraturia can also be observed in other nephropathies what induces to think in other mechanisms [18, 25].

In the very interesting work of Goraya et al. [11], they suggest that urinary citrate could be a marker of acid retention in patients without obvious metabolic acidosis, with normal serum bicarbonate levels. They analyzed in patients with arterial hypertension with CKD1 and CKD2 stages the response of acid retention and citrate excretion to prescription of an alkali-rich diet based on consumption of fruits and vegetables. They observed after this diet that acid retention and acid excretion were reduced, and that urinary citrate elimination was significantly increased in both CKD stages. Before introduction of alkaline diet, urinary citrate levels were clearly lower in CKD2 stage and even in some patients with CKD1 stage, despite having all of them normal serum bicarbonate, as occurred in our population. Urinary citrate was correlated with burden of acid retention and they estimated that each 1 mg/day increase in urinary citrate excretion was associated with a reduction of 0.096 units in acid retention.

In a very recent publication, Gianella et al. [25] analyze urinary citrate excretion in patients with chronic glomerulonephritis and describe a progressive reduction in urinary citrate as GFR declines, also showing a decrease in ammonium, sulfate, and potassium renal excretion. All these results might to be explained simply by the reduction of GFR or by a dietetic reduction in proteins or vegetables according to CKD stage. When citraturia is corrected by urinary sulfate or potassium, they observed that urinary citrate decreased accompanying to GFR reduction [25]. They also performed an acid overload test and observed that patients who showed more urinary citrate underwent less acid overload. They also suggested that urinary citrate should be considered as a marker of the compensatory mechanisms that are acting in chronic renal disease versus acid overload. These mechanisms may plausibly be a key factor that justifies our results: a progressive reduction in urinary citrate levels in ADPKD patients, starting from very early stages of CKD, even with normal bicarbonate levels, caused by an acid overload related with diet and with the progressive decrease of GFR.

We have only included serum bicarbonate levels as a very simple marker of acid–base status in analysis. We did not observe any correlation between urinary citrate and serum bicarbonate as occurred in the studies of Goraya et al. [11] and Gianella et al. [25], and did not find lower levels of serum bicarbonate among patients in the lower tertile of urinary citrate. In our data, there were few patients with really low bicarbonate levels which might explain the absence of such relationship, although we included patients with low GFR. Gianella et al. [25] also showed patients with advanced chronic renal disease without great reduction in levels of serum bicarbonate but with hypocitraturia.

If acid retention is accompanied by intracellular acidosis in tubular cells, it may also serve as stimulus for growing of renal cysts and might contribute to progressive deterioration of renal function in PKD [10]. Reduced urinary citrate levels might serve as a predictor of renal deterioration in PKD, but so far, these hypothesis has not been examined. Citrate administration in murine polycystic models has shown that growth rhythm of renal cysts and interstitial fibrosis is reduced, and glomerular filtration better preserved when sodium bicarbonate is used as alkalizing agent [26]. Different studies have shown that acidosis has a detrimental effect on CKD progression and the beneficial effects of alkaline diet or sodium bicarbonate supplements [12, 27, 29]. If patients with acid retention should be treated with sodium bicarbonate or citrate, even with normal serum bicarbonate levels, it is an interesting point of view [11].

In our study, we segmented population in tertiles of urinary citrate concentration to show the relationship between reduction of citrate and different aspects of renal function, when GFR is impaired in PKD. We found an accompanying clear reduction in urinary calcium and a mild reduction in urinary osmolality and acid uric concentration, with an increase in albuminuria and proteinuria in the lower tertile of urinary citrate. Renal excretion of citrate does not directly influence any mechanism involved in urinary concentration, in tubular handling of uric acid or calcium, or in the appearance of proteinuria. It should simply be understood that all of these aspects of renal function are altered along with GFR as PKD progresses and the growing of cysts alters vascular architecture and renal medulla. The progressive acid retention and reduced generation of ammonium due to reduction in nephronal mass would lead to reduction in urinary excretion of citrate, but also of potassium and sulfate [25]. In this sense, very reduced citrate levels in urine in one patient with similar GFR that other with higher concentrations might indicate a more damaged renal parenchyma that cannot be proven with other method. We measured TKV in part of the population of our study, given that TKV has been considered an early prognostic marker in PKD in some studies [21]. We found that urinary levels of citrate were not related with TKV when they were adjusted with GFR, like occurred with calcium and uric acid renal handling. Therefore, citrate may provide a different information as a prognostic marker in PKD.

The third important factor implicated in urinary citrate elimination is the levels of calcium in urine. We found a relationship between citrate and calcium levels in urine, with a clear reduction of calcium in each tertile of citrate. These findings coincide with those observed in normal and lithiasic population [30]. When tubular cells are exposed to an extracellular environment rich in calcium, an inhibition of NaDC-1 transporter of the apical membrane is observed, with reduction of citrate reabsorption [4, 31]. When extracellular calcium is reduced, an increase in citrate reabsorption is also observed [31]. This effect seems to be mediated in part by calcium sensing receptor [32]. In patients treated with synthetic parathyroid hormone with preserved renal function, a reduction in urinary citrate and calcium excretion can be observed, which increased again when treatment is discontinued [33]. This reduction might be actually mediated by the reduction in urinary calcium and not by parathyroid hormone. Therefore, patients with lower urinary calcium excretion due to a reduction in GFR, an increased tubular reabsorption caused by secondary hyperparathyroidism, a poor intestinal absorption, or other diverse mechanisms might cause in part an added reduction in urinary citrate in PKD.

There are several ways to express citrate levels in urine and its relation with gender can be misleading, so that it is necessary to clarify some aspects. We think that the best way to show concentrations is through urinary citrate/creatinine ratio, because it corrects from improper 24-h urine collection as it is used for calcium, uric acid, and albuminuria or proteinuria determinations. However, we must be aware that this ratio causes higher levels in women because of a lower renal excretion of creatinine than in men. On the other hand, some studies have also found higher citrate excretion in urine in females that has been attributed to a diet more profuse in vegetables and less generous in protein than in men [22, 30].

This work has several limitations that should be mentioned. We have not included any information about the diet followed by patients or medication taken. This study was conducted in a region in the south of Spain that follows, in general, a Mediterranean diet with only limitations in recommendations of taking some vegetables or some fruits when eGFR is very low (CKD4 + 5 stages) or when hyperkalemia is present. We have not registered protein intake that can contribute to acid overload and that might cause some reduction in urinary citrate. It could also be interesting to analyze impact of intake of alkaline foods (as some vegetables or fruits) in urinary citrate in different CKD stages using dietary records or with another estimation method. However, these factors will not explain the very low levels of urinary citrate found in our study. When citraturia is expressed corrected by urinary potassium (as a marker of vegetables and fruit intake), it can be observed that hypocitraturia remains, indicating that the influence of diet is a minor influence at the time to explain the low levels found in chronic renal disease. We think that diuretics can potentially have an impact when alkalosis is present, although this would increase urinary citrate levels; so in our case, it did not seem to have any influence. One last limitation is the possible influence of race in our results. Patients included in our study are hispanic white as the patients followed by Goraya et al. in their different studies [11, 12], who also found a reduction in urinary citrate when chronic renal failure progressed, suggesting that our results can probably be replicated in other populations. Thus, all these aspects mentioned will be important to consider in the design of future studies.

In summary, urinary citrate concentration is frequently found reduced in ADPKD patients with a clear correlation with the decline of GFR. The dependence of citrate renal excretion with status of acid–base metabolism in CKD recently postulated suggests that reduced levels of citrate in urine would be an expression of a progressive acid retention caused by reduction of GFR and not demonstrable with serum bicarbonate levels. These reduced levels can be discovered in patients with apparently conservated GFR and we do not know if this may indicate a worse prognosis for PKD. Urinary citrate are not related with TKV when we consider GFR. Future studies are needed to clarify if citrate can provide some extra information as a prognostic marker for the evolution of renal function in ADPKD patients.

Author contributions Research idea and study design: FJBU; data acquisition: FJBU, IHC, MVCR, EMG, and CMD; data analysis/interpretation: FJBU, IHC, and EOP; statistical analysis: FJBU. Each author contributed important intellectual content during manuscript drafting or revision. All authors approved the final version of the manuscript.

Declarations

Conflict of interest Authors have not conflict of interest to declare. We have not received any financial support for this research. We declare that the results presented in this paper have not been published previously in whole or part, except in abstract format.

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