Vasopressin in chronic kidney disease, in particular ADPKD

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Urine and plasma osmolality in patients with autosomal dominant polycystic kidney disease: reliable indicators of vasopressin activity and disease prognosis?

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Am J Nephrol. 41:248-56, 2015

Abstract

Background: Vasopressin plays an essential role in osmoregulation, but has deleterious effects in patients with ADPKD. Increasing water intake to suppress vasopressin activity has been suggested as potential renoprotective strategy. This study investigated whether urine and plasma osmolality can be used as reflection of vasopressin activity in ADPKD patients.

Methods: We measured urine and plasma osmolality, plasma copeptin concentration, total kidney volume (TKV, by MRI) and GFR (^{125}I-iothalamate). In addition, change in estimated GFR (eGFR) during follow-up was assessed.

Results: 94 patients with ADPKD were included (56 males, age 40±10 year, mGFR 77±32 ml/min/1.73m^2, TKV 1.55 (0.99 – 2.40) L. Urine osmolality, plasma osmolality and copeptin concentration were 420±195 mOsmol/l, 289±7 mOsmol/l and 7.3 (3.2 – 14.6) pmol/l, respectively. Plasma osmolality was associated with copeptin concentration (R=0.54, p<0.001), whereas urine osmolality was not (p=0.4). In addition, urine osmolality was not associated with TKV (p=0.3), in contrast to plasma osmolality (R=0.52, p<0.001) and copeptin concentration (R=0.61, p<0.001). Fifty-five patients were followed for 2.8±0.8 years. Baseline plasma and urine osmolality were not associated with change in eGFR (p=0.6 and p=0.3, respectively), whereas baseline copeptin concentration did show an association with change in eGFR, in a crude analysis (St. β= -0.41, p=0.003) and also after adjustment for age, sex and TKV (St. β= -0.23, p=0.05).

Conclusions: These data suggest that neither urine nor plasma osmolality are valid measures to identify ADPKD patients that may benefit from increasing water intake. Copeptin appears a better alternative for this purpose.

Introduction

The antidiuretic hormone arginine vasopressin (AVP) is an essential hormone for osmoregulation. When plasma osmolality increases, AVP is secreted by the pituitary gland, subsequently activating the V2 receptors of renal collecting duct cells,[1] which results in translocation of aquaporin 2 to the luminal surface of these cells, making them permeable for water.[2]

Besides the physiological stimulation of water reabsorption, AVP appears to have an essential role in the pathophysiology of Autosomal Dominant Polycystic Kidney Disease (ADPKD).[3] Animal models and a large scale phase 3 multicenter randomized controlled trial in patients with ADPKD showed that blocking the AVP V2 receptor with a V2 receptor antagonist, leads to a reduction in the rate of cyst growth and renal function loss.[4,5]

Drinking a sufficient volume of water can also reduce AVP concentration. Increasing water intake could therefore be an alternative to medical treatment with a V2 receptor antagonist to ameliorate disease progression in ADPKD. In a rat PKD model, it was indeed shown that increased water intake attenuated disease progression.[6] In ADPKD patients only one small-scale, non-randomized study has been performed, that was not able to show a favorable effect of increasing water intake.[7-9] Until other data become available, it is, based on theoretical grounds and convincing animal data, still advised that ADPKD patients should increase their water intake.[7-9] For clinicians, the question arises which ADPKD patients should increase their water intake, and what volume of fluid they should be advised to drink. In this respect, measuring urine osmolality could be of help.[3,9-11] It is generally assumed that a urine osmolality below 285 mOsmol/l, or a urine osmolality lower than plasma osmolality, reflects adequate suppression of AVP.[9,11]

ADPKD patients, however, have an impaired urine concentrating capacity, that worsens throughout their disease, presumably because they have an impaired renal medullar osmolar gradient due to cyst formation.[12] This lack of renal concentrating capacity is expected to lead to a lower urine osmolality, a higher plasma osmolality and a compensatory high level of AVP. Clinically we observed that in patients with more advanced ADPKD, urine osmolality can indeed be low, whereas AVP is high.[13] Given this observation, urine osmolality might not be a good reflection of AVP concentration in ADPKD patients, especially in those with more advanced disease.

The aim of the present study is therefore to cross-sectionally investigate in ADPKD patients whether urine osmolality and plasma osmolality are associated with AVP concentration (measured by the concentration of its surrogate plasma copeptin), and whether these associations are influenced by disease severity. Furthermore, the associations of urine and plasma osmolality as well as plasma copeptin concentration with the rate of renal function decline during follow-up are investigated.

Materials and Methods

ADPKD patients

For this study, all consecutive patients with ADPKD, aged 18-70 years, visiting our outpatient clinic from January 2007 until August 2011 were asked to participate. A diagnosis of APDKD was made based upon the revised Ravine criteria.[14] Patients were considered ineligible to participate if they received renal replacement therapy (including renal transplantation), had undergone renal surgery, were unable to undergo magnetic resonance (MR) imaging or had other diseases or conditions potentially affecting renal function (such as diabetes mellitus, pregnancy or lactation).

One hundred forty-six patients met these criteria. Thirteen patients did not give informed consent, leaving 133 patients that were scheduled for a 1-day outpatient clinical evaluation. Thirty six of these patients used diuretics and were excluded from the present analysis, because use of diuretics may influence AVP levels and urine osmolality. Three patients had plasma copeptin concentrations more than 10 times the interquartile range above the third quartile, although their plasma osmolality was within normal limits. These patients were considered outliers and their data were not taken into consideration.[15] leaving 94 patients for the cross-sectional analyses. In 55 of these patients at least one year of follow-up was available for longitudinal analyses. This study was performed in adherence to the Declaration of Helsinki, and all patients gave written informed consent.

Measurements

All patients routinely collected a 24-hour urine sample the day preceding renal function measurement. They were advised to refrain from heavy physical exercise during this urine collection. Of note, in the time period of the study (2007-2011) ADPKD patients did not receive advice on water intake. Just before renal function measurement, fasting blood samples were drawn in which creatinine (Roche enzymatic assay), plasma and urine osmolality (by freezing point depression using an Osmometer (Arkray, Kyoto, Japan), with a variation coefficient <1.0%) and copeptin were measured. Effective plasma osmolality (2 x (plasma sodium + plasma potassium) + plasma glucose) was calculated. Measurement of endogenous AVP is problematic, because AVP is unstable in isolated plasma and the available assays to measure AVP have limited sensitivity.[16] Therefore we decided to measure copeptin, a precursor of AVP, that has been shown to be a reliable marker for endogenous AVP and can be measured more reliably.[16-18] Plasma samples for copeptin measurement were immediately centrifuged at 4°C and stored at minus 80°C until the samples were thawed and measured using a sandwich immunoluminometric assay in one run on the same day (Thermo Fisher Scientific, U.S.A). The lower limit of detection was 0.4 pmol/L and the functional assay sensitivity (interassay coefficient of variation 0.20%) was 0.1 pmol.[19]

At the day of renal function measurement blood pressure was assessed during rest in supine position with an automatic device (Dinamap® G E Medical Systems, Milwaukee, Wis, USA) for 15 minutes during renal function measurement, of which the last 5 values were averaged to obtain systolic and diastolic blood pressure values. Furthermore, weight and height were determined. Body mass index was calculated as weight in kilograms (kg) divided by height in square meters. Body surface area (BSA) was calculated according to the DuBois formula.[20]

Renal function measurements were performed using the constant infusion method with ^{125}I-iothalamate to measure glomerular filtration rate (mGFR).[21,22] mGFR was normalized for BSA. After renal function measurement, the patients were followed for at least 12 months to again assess creatinine concentration to calculate the estimated Glomerular Filtration Rate (eGFR) by the Chronic Disease Epidemiology Collaboration (CKD-EPI) equation.[23] Change in eGFR during follow-up was calculated using linear regression slopes through all eGFR values (at least 2) that were available in our database. MR imaging was performed immediately after renal function measurement, using a standardised abdominal magnetic resonance imaging protocol without the use of intravenous contrast.[24] Scanning was performed on a 1.5 Tesla MR (Magnetom Avento, Siemens, Erlangen, Germany) and in 9 patients on a 3.0 Tesla MR (Intera, Philips, Best, The Netherlands). Total kidney volume (TKV) was assessed using Analyze Direct 8.0 software (AnalyzeDirect, Inc., Overland Park, KS, USA). Intra- and interreviewer coefficients of variation for TKV measurement were 2.4% and 3.1%, respectively.

Statistical Analysis

Because impaired renal function could affect the study results, baseline characteristics and all other analyses are given for the overall population as well as for participants with an mGFR > 60 ml/min/1.73m^2 and ≤ 60 ml/min/1.73m^2 separately. Parametric variables are expressed as mean ± SD, non-parametric variables as median (IQR). Differences in baseline characteristics between the two mGFR subgroups were calculated with a Chi-square test for categorical data, and for continuous data with Student's t-test or a Mann-Whitney U test in case of non-parametric data.

To investigate whether mGFR and TKV correlated with urine osmolality, plasma osmolality and copeptin concentration, the Pearson correlation coefficient was calculated. Because TKV, copeptin and urine to plasma osmolality (Uosm/Posm) ratio showed a skewed distribution, logarithmic transformation was applied to fulfill the requirement for correlation and regression analysis of normal distribution of the residuals. To visualize the associations, scatterplots were made showing the associations of mGFR and TKV with urine and plasma osmolality and with copeptin concentration. For significant associations the Deming fit regression line is depicted. In these plots patients with a mGFR > 60 ml/min/1.73m^2 and mGFR ≤ 60 ml/min/1.73m^2 are shown separately.

Furthermore univariate and multivariate regression analyses were performed to investigate whether plasma copeptin was correlated with urine osmolality, plasma osmolality, Uosm/Posm ratio, sex, age and TKV. Univariate and multivariate regression analyses were also performed to investigate whether the change in eGFR was associated with these variables. For these analyses interactions of baseline mGFR with baseline urine osmolality, plasma osmolality, Uosm/Posm and copeptin were tested.

Various sensitivity analyses were performed. Because sex influences copeptin concentration and possibly also the rate of renal function decline, interactions of sex with baseline copeptin were investigated, and the analyses were repeated stratified for sex. Analyses were also repeated including outliers of copeptin concentration. Lastly, because plasma urea concentration may rise with progressive worsening kidney function, this could distort the association between measured plasma osmolality and copeptin concentration. Therefore also the association between calculated effective plasma osmolality and copeptin concentration was investigated.

All statistical analyses were performed using SPSS 22 (SPSS Statistics, Inc., Chicago, IL, U.S.A.). A value of $p<0.05$ was considered significant and all statistical tests were 2-tailed.

Results

Patient characteristics are presented in Table 1. A total of 94 patients were included, aged 40 ± 10 years of which 59.6% were male. Most of the patients used antihypertensive medication (75.5%), on average one single class, but per protocol none of the participating patients used diuretics. There was a large spread in disease severity, with mGFR ranging from 12 to 138 ml/min/1.73m^2 and TKV from 0.47 to 10.28 L. Table 1 also shows patient characteristics stratified according to mGFR, indicating that patients with lower mGFR, as expected, were older, used more antihypertensives and had a larger total kidney volume. Furthermore, patients with lower mGFR had a lower urine osmolality, a higher plasma osmolality and a higher copeptin concentration compared to patients with mGFR > 60 ml/min/1.73m^2 (all $p<0.001$). mGFR was strongly correlated with eGFR ($R=0.9$, $p<0.001$).

Figure 1 presents the associations of urine osmolality and Uosm/Posm ratio with copeptin concentration (upper and middle panel), and shows that a considerable number of patients had a urine osmolality below 285 mOsmol/l (n=14, of which 7 with a mGFR > 60 ml/min/1.73m^2) and a Uosm/Posm ratio below 1 (n=13, of which 8 with a mGFR > 60 ml/min/1.73m^2). Table 2 gives the results of univariate and multivariate analyses with copeptin concentration as dependent variable. There was no association between urine osmolality and copeptin concentration, neither in a crude analysis nor after adjustment for age and sex. This was both the case for patients with mGFR > 60 and for patients with a mGFR ≤ 60 ml/min/1.73m^2, ($p=0.2$ and $p=0.2$, respectively). Also when urine osmolality was expressed as ratio to plasma osmolality (Uosm/Posm ratio),

no association was found with plasma copeptin concentration. This correlation again was not different in patients with mGFR > 60 compared to patients with mGFR ≤ 60 ml/min/1.73m^2 (R =0.19, p=0.2 and R = 0.26, p=0.2, respectively). Only after adjustment for mGFR and TKV, the associations between urine osmolality and Uosm/Posm ratio with copeptin concentration reached statistical significance (Table 2). In addition, we investigated the association of 24-hour urine volume with urine osmolality and copeptin concentration. No significant association was found between 24-hour urine volume and copeptin concentration (p=0.7), but 24-hour urine volume was associated with urine osmolality (R =-0.66, p<0.001).

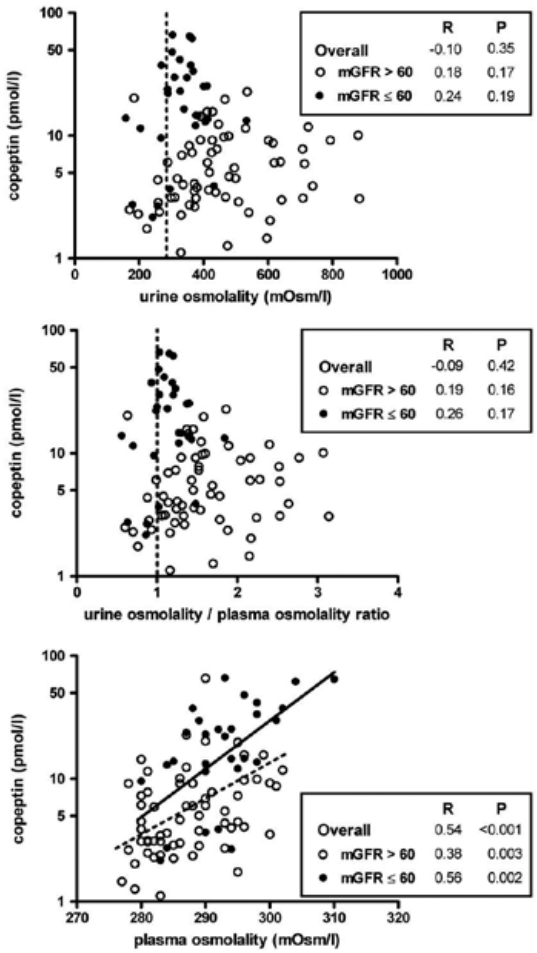

Figure 1. Association of 24-hour urine osmolality, urine to plasma osmolality ratio and plasma osmolality with copeptin concentration in ADPKD patients (overall n=94, mGFR > 60 ml/min/1.73m^2 n=64, and mGFR ≤ 60 ml/min/1.73m^2 n=30). Dashed line in upper panel represents a urine osmolality = 285 mOsmol/l, and the dashed line in the middle panel a urine osmolality equal to plasma osmolality. In the lower panel the association of plasma osmolality with copeptin concentration is shown separately for ADPKD patients with mGFR ≤ 60 ml/min/1.73m^2 (solid line) and > 60 ml/min/1.73m^2 (dashed line).

Table 1. Baseline patient characteristics

	All	Stratified according to mGFR (ml/min/1.73m²)	
		≤ 60	> 60
N	94	30	64
Age (y)	40 ± 10	47 ± 10	38 ± 9*
Male (%)	59.6	70	54.7
Body mass index (kg/m²)	25.5 ± 3.9	25.7 ± 3.1	25.4 ± 4.2
Body mass surface (m²)	2.05 ± 0.24	2.06 ± 0.25	2.03 ± 0.23
Systolic blood pressure (mmHg)	128 ± 11	130 ± 10	128 ± 12
Diastolic blood pressure (mmHg)	79 ± 9	80 ± 8	79 ± 9
Antihypertensive medication use (%)	75.5	96.7	65.6*
Plasma creatinine (umol/l)	123 ± 82	208 ± 97	82 ± 17*
Plasma osmolality (mOsmol/l)	289 ± 7	292 ± 7	289 ± 7*
Plasma copeptin (pmol/l)	7.3 (3.2 – 14.6)	19.4 (12.0 – 34.6)	4.5 (3.1 – 9.1)*
eGFR (ml/min/1.73m²)	72 ± 27	38 ± 12	90 ± 19*
mGFR (ml/min/1.73m²)	77 ± 32	38 ± 15	95 ± 18*
Urine volume (mL/24h)	2350 (1790 – 2755)	2575 (2056 – 3225)	2150 (1650 – 2650)*
Urine osmolality (mOsmol/kg)	420 ± 195	329 ± 79	459 ± 164*
Urine to plasma osmolality ratio	1.4 (1.1 – 1.8)	1.3 (1.0 – 1.3)	1.5 (1.2 – 2.1)
Total kidney volume (L)	1.55 (0.99 – 2.40)	2.20 (1.42 – 3.12)	1.36 (0.08 – 1.84)*

Parametric variables are expressed as mean ± SD, whereas non-parametric variables are given as median (interquartile range). *,p<0.05 versus group with mGFR ≤ 60 ml/min/1.73m². Abbreviations: eGFR, estimated glomerular filtration rate; mGFR, measured glomerular filtration rate

The associations of plasma osmolality with copeptin concentration are also presented in Figure 1 (lower panel). Model 1 shows that crude plasma osmolality was positively associated with copeptin concentration in the overall population (R =0.54, p<0.001), and in ADPKD patients with mGFR > 60 as well as mGFR ≤ 60 ml/min/1.73m² (R =0.4, p=0.003 and R=0.56, p=0.002, respectively). Model 2 shows that this association remained significant, when adjusted for age and sex. In Model 3, when additionally adjusted for TKV and mGFR, this association remained, although it did not reach formal statistical significance. Of note, urine osmolality and Uosm/Posm ratio were negatively associated with plasma osmolality (R =-0.22, p=0.04 and R =0.97, p<0.001, respectively).

Table 2. Multivariate linear regression analyses investigating the cross-sectional association of baseline urine osmolality, urine to plasma osmolality ratio and plasma osmolality with baseline copeptin concentration (as dependent variable) in 94 ADPKD patients.

	Model 1*		**Model 2****		**Model 3*****	
	St. β	**p-value**	**St. β**	**p-value**	**St. β**	**p-value**
Uosm	-0.10	0.35	-0.02	0.86	+0.22	0.006
Age			+0.26	0.01	-0.12	0.14
Male sex			-0.32	0.001	-0.07	0.33
mGFR					-0.66	<0.001
TKV					+0.33	<0.001
Uosm/Posm ratio	-0.09	0.42	-0.01	0.98	+0.21	0.006
Age			-0.27	0.002	-0.13	0.13
Male sex			-0.31	0.01	-0.08	0.30
mGFR					-0.66	<0.001
TKV					+0.34	<0.001
Posm	+0.54	<0.001	+0.44	<0.001	+0.18	0.07
Age			+0.09	0.36	-0.21	0.03
Male sex			-0.15	0.13	-0.10	0.26
mGFR					-0.46	<0.001
TKV					+0.30	0.004

*Model 1: crude; **Model 2: adjusted for age and sex; ***Model 3 adjusted for age, sex, mGFR and TKV. Abbreviations: St. β, standardized beta; Uosm, urine osmolality; mGFR, measured glomerular filtration rate; TKV, total kidney volume; Uosm/Posm ratio, Urine to plasma osmolality ratio; Posm, plasma osmolality.

Figure 2 shows that mGFR was significantly associated with urine osmolality, plasma osmolality and plasma copeptin concentration (all p<0.001). TKV was also significantly associated with plasma osmolality and plasma copeptin concentration (both p<0.001), but not with urine osmolality (R = -0.12, p=0.3).

Table 3 presents the associations of baseline urine osmolality, plasma osmolality, Uosm/Posm ratio and copeptin concentration with change in estimated glomerular filtration rate (eGFR) during follow-up. Fifty-five patients were followed for 2.8 ± 0.8 years and their mean change in eGFR was -3.3 ± 2.9 ml/min/1.73m^2 per year. Baseline urine osmolality was not associated with change in eGFR, neither crude, nor after adjustment for age and sex or additional adjustment for TKV. When urine osmolality was expressed as ratio of plasma osmolality, using the Uosm/Posm ratio, similar results were obtained.

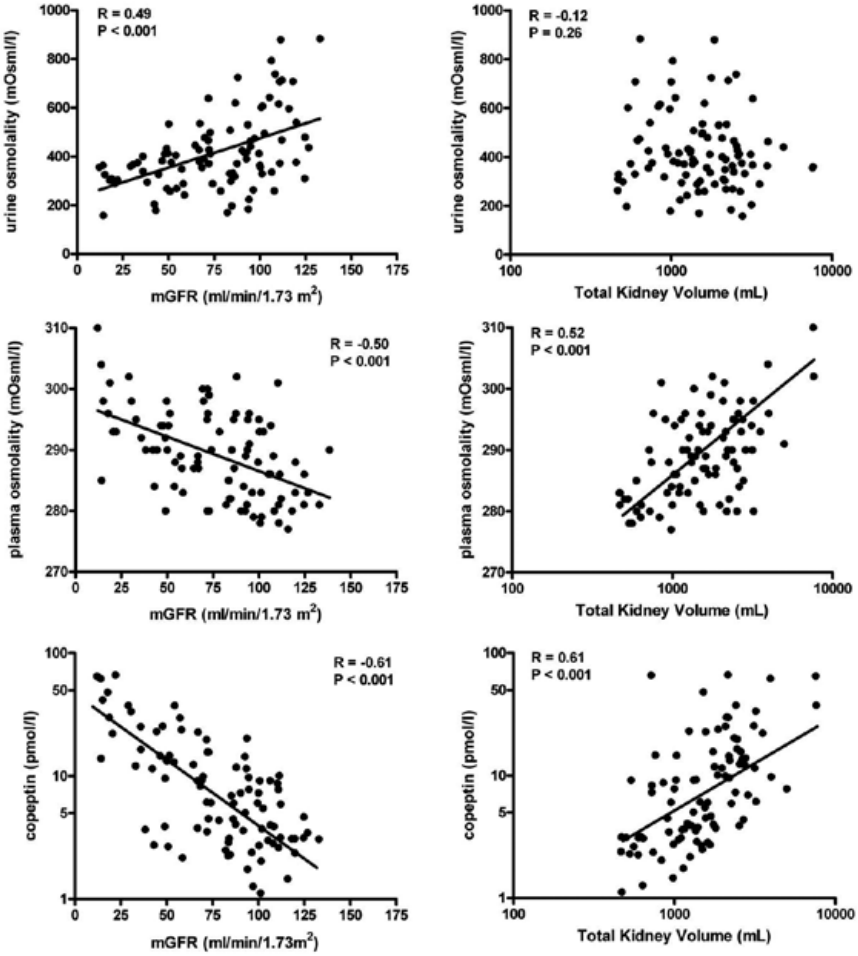

Figure 2. Associations of measured glomerular filtration rate (mGFR) and total kidney volume (log scale) with 24-hour urine osmolality, plasma osmolality and copeptin concentration in 94 ADPKD patients.

In contrast, plasma osmolality was significantly associated with decline in eGFR. However after adjustment for age and sex, only a trend was seen, and after further adjustment for TKV, the association was absent. The association of baseline copeptin concentration with change in eGFR was significant (*St.* β = -0.41, p=0.003), also after adjustment for age, sex and TKV (*St.* β = -0.23, p=0.048). In addition, we investigated the association between 24-hour urine volume with change in renal function and copeptin concentration. No significant associations were found (p=0.6 and p=0.7 respectively). Lastly, urinary sodium excretion was not correlated with change in eGFR (*R*=0.02, p=0.9).

Of note, the results of the sensitivity analyses (i.e. analyses stratified for sex and analyses including outliers of copeptin concentration) were essentially similar to the results of the primary analyses. In addition, in the multivariate regression analyses with copeptin concentration as dependent variable, interaction terms of mGFR with urine osmolality,

Table 3. Multivariate linear regression analyses investigating the association of baseline urine osmolality, urine to plasma osmolality ratio, plasma osmolality and plasma copeptin concentration with change in eGFR during follow-up (as dependent variable) in 55 ADPKD patients.

	Model 1*		Model 2**		Model 3***	
	St. β	p-value	St. β	p-value	St. β	p-value
Uosm	+0.11	0.43	+0.17	0.30	+0.14	0.34
Age			+0.10	0.54	+0.21	0.18
Male sex			+0.18	0.22	-0.06	0.71
TKV					-0.53	0.001
Uosm/Posm ratio	+0.09	0.53	+0.16	0.37	+0.13	0.40
Age			+0.09	0.59	+0.21	0.20
Male sex			+0.17	0.26	-0.04	0.78
TKV					-0.52	0.002
Posm	-0.29	0.04	-0.32	0.06	-0.11	0.55
Age			+0.11	0.49	+0.18	0.23
Male sex			+0.01	0.93	-0.09	0.56
TKV					-0.48	0.007
Copeptin	-0.41	0.003	-0.43	0.006	-0.23	0.048
Age			-0.34	0.71	+0.14	0.30
Male sex			-0.15	0.83	-0.12	0.41
TKV					-0.41	0.02

*Model 1: crude; **Model 2: adjusted for age and sex; ***Model 3 adjusted for age, sex and TKV. Abbreviations: St. β, standardized beta; Uosm, urine osmolality; TKV, total kidney volume; Uosm/Posm ratio, Urine to plasma osmolality ratio; Posm, plasma osmolality.

plasma osmolality and Uosm/Posm ratio were not significant (p=0.3, p=0.2 and p=0.6, respectively). Furthermore, for baseline copeptin concentration the interactions of sex with urine and plasma osmolality and with the Uosm/Posm ratio were not significant (p=0.3, p=0.6 and p=0.1, respectively). In addition, no significant interaction terms of copeptin with sex and mGFR were found in the analyses with change in eGFR as dependent variable (p=0.5 and p=0.4, respectively). Lastly, when calculated effective plasma osmolality was studied instead of measured plasma osmolality, essentially similar results were obtained. Effective plasma osmolality was independently associated with copeptin concentration, but lost significance after adjustment for age, sex, mGFR and TKV (*St.* β = 0.31, p=0.01; *St.* β = 0.17, p=0.1, respectively). Effective plasma osmolality was also independently associated with mGFR and TKV (*R* =-0.43, p=0.004 and *R* =0.36, p=0.002, respectively).

Discussion

Given the deleterious role of AVP in ADPKD we tried to address in the present study the question how to identify ADPKD patients with high AVP levels. In healthy persons with normal kidney function, it has been shown that AVP concentration correlates positively with urine osmolality.[18,25] In this situation, urine osmolality seems the perfect marker to monitor AVP levels. Consequently it has been suggested that in ADPKD patients a urine osmolality under 285 mOsmol/l or a urine osmolality below plasma osmolality indicates a water intake appropriate to suppress AVP levels.[9,11] However, our findings suggest that in such patients both urine osmolality and urine to plasma osmolality ratio are not appropriate to monitor AVP levels, measured as plasma copeptin concentration. Moreover, we found that urine osmolality was not associated with the rate of renal function decline during follow-up. These observations were similar in patients with impaired, as well as with relatively preserved kidney function.

A possible explanation of the fact that urine osmolality did neither correlate with copeptin levels nor with decline in renal function during follow-up, is that patients with ADPKD, even in a relatively early stage of their disease, can have an impaired urine concentration capacity. In a water deprivation test in which 15 ADPKD patients were included and 15 healthy controls, matched for sex and age, it was found that ADPKD patients had a reduced maximal urine concentration capacity compared to healthy controls, despite the fact that their GFR was still normal [26]. Early cyst formation leads to destruction of the renal architecture which, in turn, causes a failure to generate and maintain a hyperosmotic interstitial milieu, resulting in a low urine osmolality independent of vasopressin level.[12,27] The fact that the association between copeptin and urine osmolality reached significance only after correction for TKV supports this hypothesis.

Another marker to monitor activity of the AVP system might be measuring plasma osmolality. It is well known that under normal conditions, secretion of AVP is predominantly driven by an increase in plasma osmolality. In healthy persons with normal kidney function, plasma osmolality correlates therefore well with AVP levels.[18] In this study we found that in ADPKD patients plasma osmolality was indeed positively associated with copeptin concentration, although after adjustment for sex, age, TKV and mGFR, this association lost significance.

In addition, in our study plasma osmolality was only weakly associated with change in eGFR during follow-up, and this association was also lost after adjustment for covariates, indicating that measuring plasma osmolality has limited added value to predict prognosis. Again, these observations held true in patients with impaired, as well as with relatively preserved kidney function. That plasma osmolality had a limited role as marker for disease progression may be caused by the fact that plasma osmolality is usually held within narrow ranges (i.e. between 275 to 290 mOsmol/l) as variations

of only 1 to 2 percent initiate feed-back mechanisms to return osmolality to normal. Of note, measured plasma osmolality could theoretically be less reliable in case of impaired kidney function, because increases in urea concentration could influence measured plasma osmolality and thereby disturb the association of plasma osmolality with copeptin concentration. As a sensitivity analysis we therefore also analyzed the association of calculated effective plasma osmolality with copeptin concentration. Essentially similar results were obtained. We therefore consider measured plasma osmolality reliable, and used this parameter as one of our primary outcome measures.

In literature, several cohort studies have shown that TKV and AVP (measured as copeptin) are good predictors for a decline in renal function during follow-up.[28-30] Also in the present study TKV was the strongest marker for renal function decline. However, measurement of TKV is labor intensive and therefore difficult to operationalize in clinical care. In that respect measurement of copeptin concentration might be a more feasible alternative. The present study corroborates that higher copeptin is associated with more rapid renal function decline and that this associations persists after correction for age, sex, and even after additional correction for TKV. These data suggest that measurement of copeptin concentration, as alternative for measuring urine or plasma osmolality to reflect AVP activity, may be of help to identify ADPKD patients at risk for rapid disease progression.

Patients with impaired renal function had on average higher copeptin levels. However, it should be noted that copeptin concentration has a broad distribution. Some patients with impaired renal function had lower copeptin levels than the average level in patients with normal kidney function. In patients with preserved renal function the opposite can be found. Therefore, selecting patients based on GFR will not be similar as selecting patients on copeptin concentration.

It should be emphasized that this study did not investigate the role of increasing water intake on copeptin or AVP concentration, nor on the rate of disease progression. Theoretically, however, an increase in water intake is expected to reduce the rate of disease progression in ADPKD by decreasing AVP activity, as has been shown for AVP V2 receptor blockade by tolvaptan.[3] On the other hand, there may be limitations to the efficacy of increasing water intake.[8] Medical treatment with tolvaptan leads to a long-term pharmacologic suppression of the AVP pathway. It is unknown whether long-term increases in water intake can also suppress AVP activity sustainably and what volume of fluid would be necessary to achieve this. A cautionary note should be made, being that clinicians should monitor ADPKD patients with impaired renal function that increase their water intake, because these patients are at risk for overhydration and hyponatremia.

We acknowledge that this study has limitations, the main ones being that this is an observational study and that most associations are based upon cross-sectional data. Our findings should therefore be considered as hypothesis generating. Secondly, a

relatively small number of patients was included. That we despite this limitation found a significant association between copeptin concentration and change in kidney function indicates that our data are robust. Of note, this number of patients did also not allow analyses stratified for all CKD stages, and we therefore analyzed our data for participants stratified for mGFR > and ≤ 60 ml/min/1.73m^2. Lastly, in the participants with a ≤ 60 ml/min/1.73m^2 the majority of patients was male, which potentially could influence the study results. However, our study results did not change essentially in sex stratified analyses, and sex did not appear to be a significant effect modifier. Strengths of our study are that this is the first study that investigates in ADPKD patients the associations between plasma copeptin concentration, plasma and urine osmolality at baseline, and the associations of these variables with change in kidney function during follow-up. Moreover, we investigated whether these associations depend on disease severity in ADPKD. Furthermore, we assessed GFR and TKV at baseline using gold standard measures.

In conclusion, our data suggest that plasma and urine osmolality cannot be used to identify ADPKD patients with a high copeptin (i.e. vasopressin) concentration that are at risk for a more rapid rate of kidney function decline during follow-up. Urine and plasma osmolality seem therefore no valid measures to identify ADPKD patients with a worse prognosis. For this purpose measuring copeptin concentration may be a better alternative.

Disclosures

JS is an employee of ThermoFisher Scientific, the company that manufactures and holds patent rights on the copeptin assay. The other authors declared no competing interests.

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