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Uroflowmetry alterations in patients with autosomal dominant polycystic kidney disease

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Abstract. – OBJECTIVE: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a heterogeneous inherited disease characterized by renal and extrarenal manifestations with progressive fluid-filled cyst development leading to end-stage renal disease. Our aim was to evaluate the prevalence of obstructive urological disease in ADPKD patients and possible associations with endothelial dysfunction, nutritional, metabolic and inflammatory markers.

PATIENTS AND METHODS: The study included ADPKD patients and control group, who carried out uroflowmetry, an assessment of renal function, metabolic and nutritional parameters and an evaluation of endothelial dysfunction and atherosclerotic markers, such as Renal Resistive Index (RRI), Intima-Media Thickness (IMT) and Flow-Mediated Dilation (FMD).

RESULTS: We enrolled 37 ADPKD patients (20 males with 51.0 ± 14.3 years) and 34 control group (18 males with 60.7 ± 14.4 years). We showed a significant reduction in Max Flow Rate (Qmax) ($p \leq 0.001$), age ($p = 0.006$), FMD ($p = 0.023$) and Voiding Volume ($p = 0.053$), in addition to a significant increase in Voiding Time and Diastolic Blood Pressure ($p \leq 0.001$, $p = 0.049$; respectively) in ADPKD patients with respect to control group. Moreover, we found a negative correlation between Qmax and creatinine (r= -0.44, $p = 0.007$), RRI (r= -0.49, $p \leq 0.001$) and intact Parathyroid Hormone (r = -0.329, $p = 0.046$), while we found a positive correlation between Qmax and MDRD (r = 0.327, $p = 0.048$) and between Voiding Time and serum uric acid (r= 0.34, $p = 0.039$) in ADPKD patients with respect to control group.

CONCLUSIONS: In our study, we showed an elevated prevalence of urological functional diseases in ADPKD patients; therefore, we suggest to include uroflowmetry in the assessment of these patients, considering the non-invasiveness, repeatability and low cost of the exam. An early intervention could slow down the progression of renal damage and an early screening of the main cardiovascular risk factors could reduce the high morbidity and mortality in ADPKD patients.

Key Words:
Autosomal dominant polycystic kidney disease, Chronic kidney disease, Urological disorders, Uroflowmetry, Max flow rate, End stage renal disease.

Introduction

The autosomal dominant polycystic kidney disease (ADPKD) is a monogenic hereditary disease and it is a systemic disorder which causes the development of cysts in kidneys and in other areas of the body, leading to many clinical manifestations both renal and extrarenal. The genes involved in this pathology are PKD1 and PKD2, whose loci are located on chromosome 16 and on chromosome 4, respectively[1]. These genes encode two proteins, polycystin-1 (PC1) and polycystin-2 (PC2), with control functions on proliferation, polarization, differentiation, and secretion of fluids in renal tubular cells. Recently the PKD3 gene, called GANAB, was also discovered[2,3]. Renal manifestations of ADPKD include many types of disorders as urinary tract infections, kidney stones and hematuria, while extrarenal manifestations can include pain, hypertension, left ventricular hypertrophy, hepatic cysts, intracranial aneurysm, diverticulosis and abdominal and inguinal hernias[4,5]. ADPKD is associated with different alterations, not only related to

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the formation of cysts, but also to a possible abnormal metanephric differentiation with possible dysplasia or ureteropelvic atresia, as reported by Kobayashi et al[6]. Thus, we aimed at evaluating the prevalence of an obstructive urological disease in ADPKD patients and possible associations with endothelial dysfunction, nutritional, metabolic and inflammatory markers.

Patients and Methods

Patients

We performed an observational, controlled, cross-sectional study on 71 patients, 37 ADPKD patients and 34 control group matched by sex and estimated glomerular filtration rate (eGFR), at the University Hospital "Policlinico Umberto I" of Rome, Sapienza University of Rome, Italy. Patients were enrolled from September 2015 to June 2017. The investigation was approved by the Local Clinical Research Ethics Committee with protocol no. 3169/15. This research was conducted in accordance with the principles outlined in the Declaration of Helsinki and the written consent was obtained from each patient enrolled. Participants were divided into 2 groups, ADPKD patients and control group, including CKD patients, comparable by gender and eGFR.

Inclusion Criteria

Patients aged > 18 years with ADPKD and control group with CKD.

ADPKD was defined according to the Pei's criteria[7]. The eGFR was calculated with the abbreviated Chronic Kidney Disease Epidemiology formula (CKD-EPI), as defined by Levey et al[8].

Exclusion Criteria

We recorded the cardiovascular history and excluded patients affected by heart failure, neoplastic diseases and acute coronary syndrome within three months before the study.

We excluded also patients with known urinary abnormalities suggestive of concomitant glomerular disease and patients who refused to give consent as well as patients with missing data.

Laboratory Measurements

In all patients, the levels of fasting plasma glucose (mg/dL), insulin (μU/mL), total serum cholesterol (mg/dL), triglycerides (mg/dL), high-density lipoprotein (HDL) (mg/dL), creatinine (mg/dL), serum nitrogen (mg/dL), serum uric acid (SUA) (mg/dL), fibrinogen (mg/dL), calcium (mg/dL), phosphorus (mg/dL), serum electrolytes (mEq/L), C-reactive protein (CRP) (μg/L), homocysteine (Hcy) (μmol/L) were measured using standard automated techniques. LDL-cholesterol was calculated using the Friedewald equation: LDL (mg/dL) = total cholesterol − HDL − (triglycerides/5). Parathyroid Hormone was measured using a two-site assay which measures "intact" hormone (iPTH) (pg/ml) and 25-hydroxyvitamin D (25-OH-VitD) (ng/mL) was measured by radioimmunoassay. Serum albumin (g/dL) was determined by the bromocresol purple method. Microalbuminuria and proteinuria 24 h were carried out.

Anthropometric Assessments

Body weight was determined to the nearest 0.1 kg using a calibrated digital scale. Body mass index was calculated from the patient's weight and height (weight (kg)/[height (m)]^2).

Blood Pressure Measurements

Blood pressure (BP) measurements were made using a standard automatic sphygmomanometer with cuffs adapted to the arm circumference, as reported by the guidelines (9). Hypertension was defined as Systolic Blood Pressure (SBP) ≥ 140 mmHg or Diastolic Blood Pressure (DBP) ≥ 90 mmHg on repeated measurements. We have calculated the Ankle/Brachial Index (ABI), the ratio of the SBP in the ankle and in the arm (normal values 0.9-1)[10].

Echocardiography

All patients underwent transthoracic echocardiography with a cardiovascular ultrasound system (Vivid E9, GE VINGMED ULTRASOUND A/S, Strandpromenaden 45, N-3191 Horten, GE, Norway). Measurements of cardiac chambers were made according to guidelines[11,12]. Left ventricular ejection fraction and mass index by modified biplane Simpson's method were estimated. Peak early (E) and late (A) diastolic velocities, deceleration time, left ventricular isovolumic relaxation time and the myocardial performance index were obtained using standard Doppler practices. Standard parasternal, apical and subcostal views have been used.

Carotid Intima-Media Thickness Assessment (IMT)

Participants were evaluated with the high-resolution B-mode ultrasound machine Toshiba Aplio xV (Toshiba Aplio xV, Toshiba America Medical Systems, Inc., Tustin, CA, USA) equipped with a

5 to 12 MHz linear transducer, following a standardized protocol[13]. IMT was measured at three points on the far walls of both left and right distal common carotid arteries and the mean IMT was calculated as the average IMT on both sides. The IMT value was considered normal between 0.55 and 0.9 mm[14].

Flow-Mediated Dilation Brachial Artery (FMD)

According to the method described by Celermajer and others (15), the endothelium-dependent vasodilation of the brachial artery was assessed using a B-mode ultrasound machine Toshiba Aplio xV (Toshiba Aplio xV, Toshiba American Medical Systems, Inc., Tustin, CA, USA) equipped with a 5 to 12 MHz linear transducer, following a standardized protocol (16). The flow-mediated-dilation (FMD) was typically expressed as a change in the post-stimulus diameter and as a percentage of the baseline diameter.

FMD: (diameter post-hyperemia-basal diameter/basal diameter) x 100.

The values of FMD were considered normal if they were greater than 10%.

Renal Resistive Index (RRI)

Participants were studied with the high-resolution B-mode ultrasound machine Toshiba Aplio xV (Toshiba Aplio xV, Toshiba American Medical Systems, Inc., Tustin, CA, USA) equipped with a 3-3.5 MHz convex transducer. Renal resistive index (RRI) values were determined with the mean of three separate measurements in the superior renal pole, regional interpolar and lower pole at the level of the interlobar, interlobular or arcuate arteries in both kidneys. We used an anterior and an oblique approach, to detect renal arteries and intra-parenchymal vessels, and we used a posterior approach with adjustment of direction if the cystic lesions were too large and did not permit a clear view. Three to five reproducible and consecutive waveforms with similar aspect from each kidney were obtained. These measurements were used to calculate the average RRI value for each kidney, and then, the average RRI value for each patient was calculated as the mean of the RRI in the left and right kidney[17]. We determined the peak systolic velocity and end-diastolic velocity (centimeters/second) to calculate the RRI as = [1-(end-diastolic velocity ÷ maximal systolic velocity)] x 100 (18). The intra-reader correlation coefficient for RRI was 0.97, whereas the inter-reader was 0.92.

Uroflowmetry

All patients have carried out an uroflowmetry, with a commercially available instrument (Dantec Medical®, the Dan Flow 1100-WiFi version; Dantec Dynamics Ltd, a Nova Instruments Company, Garonor Way, Royal Portbury, Bristol BS20 7XE United Kingdom), evaluating Flow Max Rate (Qmax) (20 < normal value < 35 ml/s), Voiding Time (normal value < 20 s) and Voided Volume (normal value > 150 ml) values[11]. The urodynamic examination is a tool to evaluate the pressure-flow relation between the bladder and the urethra to assess the functional status of the lower urinary tract. The main goal of the urodynamic evaluation is to aid the urologist in the correct diagnosis of the lower urinary tract dysfunction based upon its pathophysiology[19]. Urodynamic studies should assess the filling and storage phase, as well as the voiding phase of the bladder and urethral function. Simple urodynamic tests involve performing noninvasive uroflow studies, obtaining a post-void residual (PVR) urine measurement, the amount of residual urine in the bladder after a voluntary void, and the performing single-channel cystometrography (CMG). Currently, the normal values of the PVR are poorly defined. However, most urologists agree that volumes from of 50 mL to 100 mL constitute the lower threshold defining an abnormal residual urine volume[20]. CMG is the graphic recording of the pressure exerted at varying degrees of filling of the urinary bladder and it measures the contractile force of the bladder in the voiding phase. A single-channel CMG is used to assess the first sensation of filling, fullness, and urinary urge. Filling CMG measures the detrusor muscle function and the intra-abdominal pressure, while voiding CMG measures the detrusor muscle contractility and the detection of any obstructions. During this phase, the bladder compliance and the evaluation of detrusor contractions can also be noted[21].

Statistical Analysis

Data were analyzed using the STATA software. The normality of the variables was tested using the Shapiro-Wilk method for normal distributions. Continuous normal variables were expressed as mean ± the standard deviation of all. The Student-U or the Mann-Whitney *t*-test were used to determine the difference between groups. The bivariate correlations and the degree of association between variables were

obtained by the Spearman test. A value of p <0.05 was considered statistically significant.

Results

The study included 37 consecutive ADPKD patients (20 males) with a mean age of 51.02 ± 14.35 years, and 34 control group (18 males) with a mean age of 60.76 ± 14.41 years. Population characteristics are shown in Table I. There were no significant differences between the two groups regarding SBP and eGFR (Table I). On the contrary, we reported a significant reduction in Qmax ($p \leq 0.001$) (Figure 1), age ($p = 0.006$) and FMD ($p = 0.023$), with a reduction in Voiding Volume ($p = 0.053$) (Table I) and a significant increase both in Voiding Time (Figure 2) and DBP (Table I) ($p \leq 0.001$, $p = 0.049$, respectively) in ADPKD patients with respect to control group. Moreover, we found a significant negative correlation between Qmax and creatinine (r = -0.44, $p = 0.007$), RRI (r = -0.49, $p \leq 0.001$) and iPTH (r = -0.329, $p = 0.046$) (Figure 3), while we found a significant positive correlation between Qmax and MDRD (r = 0.327, $p = 0.048$) (Figure 4) and between Voiding Time and Serum Uric Acid (r = 0.340, $p = 0.039$) in ADPKD patients with respect to control group.

Discussion

Polycystic kidney disease includes a series of inherited disorders which determine the cyst development in the kidney as well as a series of systemic manifestations as ADPKD and autosomal recessive PKD (ARPKD). However, there are many other syndromes such as Meckel, Joubert,

Table I. Patient's characteristics. Data are shown as mean ± standard deviation.

Parameters	ADPKD (n=37)	Control Group (n=34)	*p*-value
Age	51.02±14.35	60.76±14.41	**0.006**
SBP	134.85 ±16.67	131.62±18.82	0.448
DBP	83.10 ± 11.86	78.38 ± 7.25	**0.049**
Creatinine	1.50±0.72	1.60±0.70	0.591
eGFR	52.45 ±23.0	45.14 ±18.26	0.145
Voiding volume	281.35 ±138.25	349.23 ±151.90	0.052
Voiding time	31.54±14.86	20.56± 10.94	**≤0.001**
FMD	9.58±6.4	12.47±3.9	**0.023**
Qmax	22.10±13.62	30.08± 11.39	**≤0.001**

Abbreviations: SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; eGFR, estimated Glomerular Filtration Rate; FMD, Flow Mediated Dilation; Qmax, Max Flow Rate.

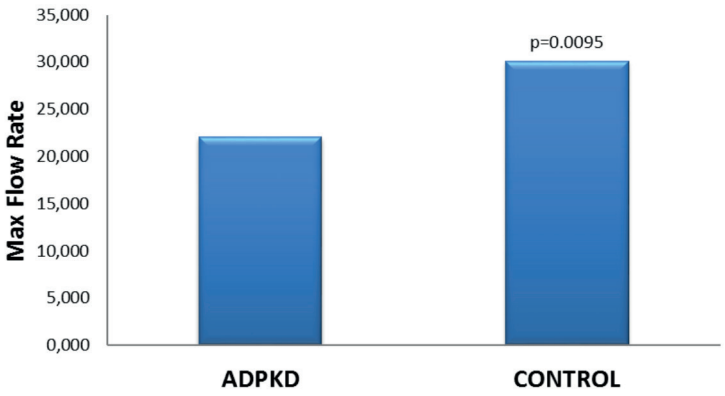

Figure 1. Bar chart. Mean value of the Max flow rate is significantly reduced in ADPKD Group with respect to control group (22.10 ± 13.67 *vs.* 30.08 ± 11.30, $p = 0.009$). Boxes represent the averages; Abbreviations: ADPKD, Autosomal dominant polycystic kidney disease.

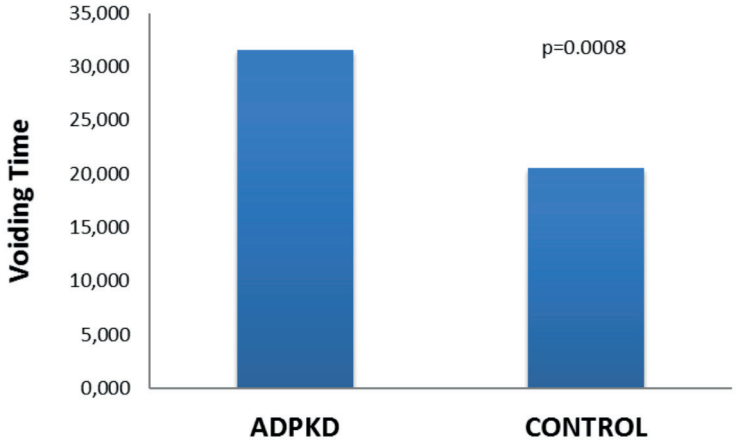

Figure 2. Bar chart. Mean value of Voiding time is significantly higher in ADPKD Group with respect to control group (31.54 ± 14.86 *vs.* 20.55 ± 10.94, $p = 0.0008$). Boxes represent the averages; Abbreviations: ADPKD, Autosomal dominant polycystic kidney disease.

Bardet-Biedl and tuberous sclerosis, which can occur with cystic phenotype[2]. In addition to PKD1, PKD2, and PKD3, the main known genes involved in the cystic phenotype are Hepatocyte nuclear factor-1-beta (HNF1ß) (associated with the renal cysts and diabetes syndrome), PKHD1 (gene involved in the production of a protein called fibrocystin). Furthermore, some interbreeding of conditional PKD1 or PKD2 mouse models have suggested additive cistogenic effects associated with mutations of more than one cystogen determining different pathology form[2,22]. Primary cilia are crucial in the pathogenesis of ciliopathy, in fact, the development of cysts results from cilia loss and PC reduction in the mammalian[23]. The relation between cilia and PKD is best understood in the syndromic ciliopathies[24], but the precise function of the PC complex on the cilium is still an unresolved problem. PC1 and PC2 are the polycystins regulating the cilia Ca^{2+} compartment, moreover, changes in the cilium can have global cytoplasmic effects. Some studies[2,25] showed that the PC complex could intervene in regulating cell division. Moreover, a direct role of PC in the vascular disease associated with ADPKD, and the increased cardiovascular risk, has been suggested

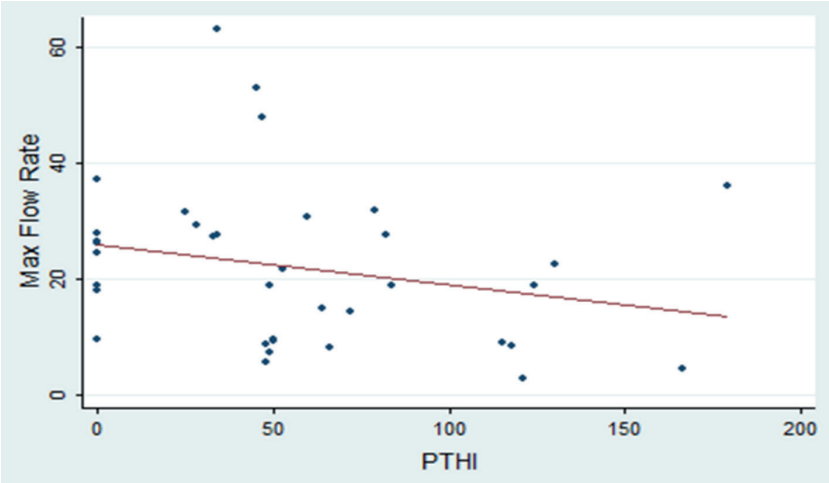

Figure 3. Linear regression graph. Correlation between Max flow rate and PTHi ($r = -0.329$ $p = 0.046$) in ADPKD patients. Abbreviations: ADPKD, Autosomal dominant polycystic kidney disease; PTHi, Parathyroid Hormone.

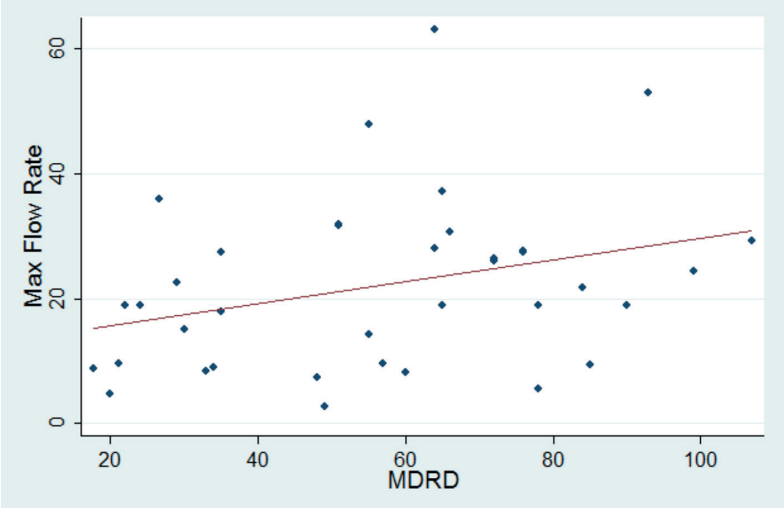

Figure 4. Linear regression graph. Correlation between Max flow rate and MDRD (r = 0.327, p = 0.048) in ADPKD patients. Abbreviations: MDRD, Modification of Diet in Renal Diseases.

by murine models[26]. PC1 and PC2 are fundamental for the differentiation of the tubular epithelium during nephrogenesis. An impaired apoptosis accompanies the increased cell proliferation in polycystic kidneys[27-29], in fact an imbalance favoring proliferation over apoptosis contributes to the development of cysts, microscopic adenomas and epithelial hyperplasia in PKD[30-31]. Many genes controlling proliferation and apoptosis during the embryonic development[32-34] and tissue regeneration also control cystogenesis in PKD resulting in persistent expression of developmental genes normally downregulated in mature kidneys and in the failure to suppress cell proliferation[35,36]. Epidermal growth factor family (EGF) (EGF, Transforming growth factor alpha, heparin-binding EGF, and amphiregulin), hepatocyte growth factor (HGF), insulin-like growth factor (IGF1), and their tyrosine kinase receptors, ErbB1 to ErbB4, MET, and IGF1R, that regulate ureteric bud branching and collecting duct elongation, in late stages of nephrogenesis[37-42], in addition to promoting tubular regeneration after renal injury[43-49], could play a role in PKD pathogenesis. In our study, ADPKD patients showed a significantly reduced Qmax and Voiding volume with a significantly higher Voiding time compared to control group, showing the presence of urological abnormalities. As reported by Kobayashi et al[6], the polycystic diseases could be associated with different alterations, including a potential abnormal differentiation of metanephros with possible dysplasia or ureteropelvic atresia. The site and the degree of narrowing in the infundibulopelvic system produce various congenital anomalies such as hydronephrosis and calyceal diverticulum, and also ureteropelvic junction stenosis[50,51]. In fact, there are polymorphic markers such as 3'-HVR, SM-7, KG-8, and CW3 that map near the locus PKD1 and the locus of tuberous sclerosis (TSC-2) on chromosome 16[52,53]. These anomalies could be part of a series of obstructive dysplastic renal conditions, characterized by an inherited autosomal dominant transmission, with variable expressivity[54-58]. A study conducted on patients with polythelia showed that accessory breast tissue can be associated with congenital and hereditary abnormalities of the kidneys and the urinary tract including ADPKD, cystic renal dysplasia, congenital stenosis of the pieloureteral junction, suggesting a syndromic manifestation, with the probable autosomal dominant transmission[59]. Another hypothesis which could explain a greater incidence of urological alterations, reported also from Wetzel et al[60], is a greater risk of urinary infection found in ADPKD patients that could increase the risk of obstructive pathologies. Gao et al[61] reported that the cellular and molecular mechanisms responsible for the high urinary tract infection (UTI) incidence in ADPKD patients remain unknown, and he showed that α-intercalated cells (α-ICs) of the collecting ducts function in the innate immune defense against UTI, inhibiting bacterial growth by acidifying urine and secreting neutrophil gelatinase-associated lipocalin (NGAL) which chelates siderophore-containing iron, suggesting that

ADPKD patients with recurrent UTI could have a reduced number and/or impaired function of α-ICs. Symptomatic lower UTI affects 50-75% of all ADPKD patients, nearly 30-50% of patients with ADPKD will have a UTI, either pyelonephritis or cyst infection, during their lifetime[62]. This work showed a positive correlation between Qmax and MDRD, suggesting a possible role of the obstructive pathology in the progression of renal failure. Age is also significantly reduced in ADPKD patients compared to control group, excluding more frequent obstructive diseases of older adults such as benign prostatic hypertrophy. Furthermore, our study showed a positive correlation between Voiding time value and serum uric acid and a negative correlation between Qmax and iPTH value in ADPKD patients. The serum uric acid and iPTH are associated with an increased cardiovascular risk. The increase in iPTH, usually associated with vitamin D deficiency, leads to an increased cardiovascular risk, altering cardiomyocytes and vascular smooth muscle cells determining the left ventricular hypertrophy[63]. Hyperuricemia could play a causal role in the oxidative stress, inflammation, and atherosclerosis; as demonstrated in several controlled and randomized studies, treatment with allopurinol resulted in an improvement of oxidative stress and endothelial function[64,65]. Moreover, Qmax was negatively associated with the RRI value. The VPS-VTD/VPS ratio, called RRI, is considered one of the most sensitive tools in the study of medical nephropathies, allowing the quantification of changes in the renal plasma flow. The value of RRI can be considered a marker of renal damage progression[66], in fact, a value of RRI > 0.70 can be considered an independent risk factor of worsening of renal function in CKD[67]. Furthermore, our study showed that DBP is significantly higher in ADPKD patients than in control group. In ADPKD, hypertension is an early condition occurring in 60% of patients before the renal function is impaired[67]. The remodeling of the arterial wall is crucial in the progression of hypertension inducing an increase in peripheral resistance, especially in small-caliber vessels. These structural changes can induce a reduction in vascular compliance, an increase in arterial stiffness and an increase in vascular resistance[68]. We also found a significant difference in the FMD between the two groups. Endothelial dysfunction has a central role in the pathogenesis of cardiovascular disease[69] and it is mainly due to an alteration of the endocrine-paracrine endothelium activity[70], characterized by insufficient endothelium-dependent vasodilation. According to Wang et al[71] and Kocaman et al[72], the endothelial dysfunction is present in both hypertensive and normotensive patients with ADPKD. The limitations of our study are the relatively small cohort of CKD and ADPKD patients and the cross-sectional single-center design. Moreover, a significant proportion of CKD patients were on several medications with a potential impact on different indices which may have possibly confounded the results. Moreover, it is based on associations rather than on a causality relation and therefore it needs further prospective follow-up studies with a larger number of patients and stronger endpoints to show causality.

Conclusions

We showed an elevated prevalence of urological diseases in ADPKD patients, therefore we suggest to insert the uroflowmetry in the assessment of these patients, considering the non-invasiveness, repeatability and low cost of the exam. Early intervention, whenever possible, could slow down the progression of kidney damage. Moreover, we suggest a screening of the main cardiovascular risk factors to reduce the high morbidity and mortality of ADPKD patients.

Declaration of interest

The authors have declared that they have no conflict of interests. All procedures performed in studies involving human participants were in accordance with the ethical standards of University Hospital "Policlinico Umberto I" of Rome, Sapienza University of Rome, Italy and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Institutional Review Board approval has been obtained. Informed consent was obtained from all individual participants included in the study. The authors are responsible for the content and writing of the paper. The manuscript has been seen and approved by all authors. This study was not funded. The manuscript is not under consideration for publication elsewhere.

References

1) Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet 2007; 369: 1287-1301.

2) Harris PC, Torres VE. Genetic mechanisms and signaling pathways in autosomal dominant polycystic kidney disease. J Clin Invest 2014; 124: 2315-2324.

3) Hateboer N, v Dijk MA, Bogdanova N, Coto E, Saggar-Malik AK, San Millan JL, Torra R, Breuning M, Ravine D. Comparison of phenotypes of polycystic kidney disease types 1 and 2: European PKD1-PKD2 Study Group. Lancet 1999; 353: 103-107.

4) Grantham JJ. Clinical practice. Autosomal dominant polycystic kidney disease. N Engl J Med 2008; 359: 1477-1485.

5) Srivastava A, Patel N. Autosomal dominant polycystic kidney disease. Am Fam Physician 2014; 90: 303-307.

6) Kobayashi M, Kaplan BS, Bellah RD, Sartore M, Rappaport E, Steele MW, Mansfield E, Gasparini P, Surrey S, Fortina P. Infundibulopelvic stenosis, multicystic kidney, and calyectasis in a kindred: clinical observations and genetic analysis. Am J Med Genet 1995; 59: 218-224.

7) Pei Y, Obaji J, Dupuis A, Paterson AD, Magistroni R, Dicks E, Parfrey P, Cramer B, Coto E, Torra R, San Millan JL, Gibson R, Breuning M, Peters D, Ravine D. Unified criteria for ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol 2009; 20: 205-212.

8) Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F; Chronic Kidney Disease Epidemiology Collaboration. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 2006; 145: 247-254.

9) Williams B, Poulter NR, Brown MJ, Davis M, McInnes GT, Potter JF, Sever PS, Thom SM. BHS guidelines working party, for the British Hypertension Society. British Hypertension Society guidelines for hypertension management 2004 (BHS-IV): summary. BMJ 2004; 328: 634-640.

10) Price JF, Tzoulaki I, Lee AJ, Fowkes FG. Ankle brachial index and intima media thickness predict cardiovascular events similarly and increased prediction when combined. J Clin Epidemiol 2007; 60: 1067-1075.

11) Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440-1463

12) Lai S, Coppola B, Dimko M, Galani A, Innico G, Frassetti N, Mariotti A. Vitamin D deficiency, insulin resistance, and ventricular hypertrophy in the early stages of chronic kidney disease. Ren Fail 2014; 36: 58-64.

13) Ho CY, Solomon SD. A clinician's guide to tissue oppler imaging. Circulation 2006; 113: 396-398.

14) Lai S, Dimko M, Galani A, Coppola B, Innico G, Frassetti N, Mazzei ED, Mariotti A. Early markers of cardiovascular risk in chronic kidney disease. Ren Fail 2015; 37: 254-261.

15) Patel S, Celermajer DS. Assessment of vascular disease using arterial flow mediated dilatation. Pharmacol Rep 2006; 58: 3-7.

16) Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R; International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force. J Am Coll Cardiol 2002; 39: 257-265.

17) Lai S, Ciccariello M, Dimko M, Galani A, Lucci S, Cianci R, Mariotti A. Cardio-renal syndrome type 4: the correlation between cardiorenal ultrasound parameters. Kidney Blood Press Res 2016; 41: 654-662.

18) Radermacher J, Chavan A, Bleck J, Vitzthum A, Stoess B, Gebel MJ, Galanski M, Koch KM, Haller H. Use of Doppler ultrasonography to predict the outcome of therapy for renal artery stenosis. N Engl J Med 2001; 344: 410-417.

19) Kelly CE. Evaluation of voiding dysfunction and measurement of bladder volume. Rev Urol 2004; 6: 32-37.

20) Lee H, Kim KB, Lee S, Lee SW, Kim M, Cho SY, Oh SJ, Jeong SJ. Urodynamic assessment of bladder and urethral function among men with lower urinary tract symptoms after radical prostatectomy: A comparison between men with and without urinary incontinence. Korean J Urol 2015; 56: 803-810.

21) Abdul-Rahman A, Al-Hayek S, Belal M. Urodynamic studies in the evaluation of the older man with lower urinary tract symptoms: when, which ones, and what to do with the results. Ther Adv Urol 2010; 2: 187-194.

22) Fedeles SV, Tian X, Gallagher AR, Mitobe M, Nishio S, Lee SH, Cai Y, Geng L, Crews CM, Somlo S. A genetic interaction network of five genes for human polycystic kidney and liver diseases defines polycystin-1 as the central determinant of cyst formation. Nat Genet 2011; 43: 639-647.

23) Yoder BK, Hou X, Guay-Woodford LM. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia. J Am Soc Nephrol 2002; 13: 2508-2516.

24) Garcia-Gonzalo FR, Reiter JF. Scoring a backstage pass: mechanisms of ciliogenesis and ciliary access. J Cell Biol 2012; 197: 697-709.

25) Ma M, Tian X, Igarashi P, Pazour GJ, Somlo S. Loss of cilia suppresses cyst growth in genetic models of autosomal dominant polycystic kidney disease. Nat Genet 2013; 45: 1004-1012.

26) Qian Q, Hunter LW, Li M, Marin-Padilla M, Prakash YS, Somlo S, Harris PC, Torres VE, Sieck GC. PKD2 haploinsufficiency alters intracellular calcium in vascular smooth muscle cells. Hum Mol Genet 2003; 12: 1875-1880.

27) Woo D. Apoptosis and loss of renal tissue in polycystic kidney diseases. N Engl J Med 1995; 333: 18-25.

28) Goilav B. Apoptosis in polycystic kidney disease. Biochim Biophys Acta 2011; 1812: 1272-1280.

29) Li X, Guo M, Shao Y. Ultrastructural observations of programmed cell death during metanephric development in mouse. Microsc Res Tech 2013; 76: 467-475.

30) Basile DP, Liapis H, Hammerman MR. Expression of bcl-2 and bax in regenerating rat renal tubules following ischemic injury. Am J Physiol 1997; 272: 640-647.

31) Gregoire JR, Torres VE, Holley KE, Farrow GM. Renal epithelial hyperplastic and neoplastic proliferation in autosomal dominant polycystic kidney disease. Am J Kidney Dis 1987; 9: 27-38.

32) Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumormetastasis. Dev Cell 2008; 14: 818-829.

33) Schäfer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol 2008; 9: 628-638.

34) Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010; 11: 834-848.

35) Cowley BD JR, Chadwick LJ, Grantham JJ, Calvet JP. Elevated proto-oncogene expression in polycystic kidneys of the C57BL/6J (cpk) mouse. J Am Soc Nephrol 1991; 1: 1048-1053.

36) Calvet JP. Polycystic kidney disease: primary extracellular matrix abnormality or defective cellular differentiation? Kidney Int 1993; 43: 101-108.

37) Cybulsky AV, Goodyer PR, McTavish AJ. Epidermal growth factor receptor activation in developing rat kidney. Am J Physiol 1994; 267: F428-F436.

38) Zhang Z, Pascuet E, Hueber PA, Chu L, Bichet DG, Lee TC, Threadgill DW, Goodyer P. Targeted inactivation of EGF receptor inhibits renal collecting duct development and function. J Am Soc Nephrol 2010; 21: 573-578.

39) Ishibe S, Karihaloo A, Ma H, Zhang J, Marlier A, Mitobe M, Togawa A, Schmitt R, Czyczk J, Kashgarian M, Geller DS, Thorgeirsson SS, Cantley LG. Met and the epidermal growth factor receptor act cooperatively to regulate final nephron number and maintain collecting duct morphology. Development 2009; 136: 337-345.

40) Moerth C, Schneider MR, Renner-Mueller I, Blutke A, Elmlinger MW, Erben RG, Camacho-Hübner C, Hoeflich A, Wolf E. Postnatally elevated levels of insulin-like growth factor (IGF)-II fail to rescue the dwarfism of IGF-I-deficient mice except kidney weight. Endocrinology 2007; 148: 441-451.

41) Takemura T, Hino S, Okada M, Murata Y, Yanagida H, Ikeda M, Yoshioka K, Harris RC. Role of membrane-bound heparin-binding epidermal growth factor-like growth factor (HB-EGF) in renal epithelial cell branching. Kidney Int 2002; 61: 1968-1979.

42) Rogers SA, Powell-Braxton L, Hammerman MR. Insulin-like growth factor I regulates renal development in rodents. Dev Genet 1999; 24: 293-298.

43) Sakurai H, Tsukamoto T, Kjelsberg CA, Cantley LG, Nigam SK. EGF receptor ligands are a large fraction of *in vitro* branching morphogens secreted by embryonic kidney. Am J Physiol 1997; 273: F463-472.

44) Tang J, Liu N, Zhuang S. Role of epidermal growth factor receptor in acute and chronic kidney injury. Kidney Int 2013; 83: 804-810.

45) Zhou D, Tan RJ, Lin L, Zhou L, Liu Y. Activation of hepatocyte growth factor receptor, c-met, in renal tubules is required for renoprotection after acute kidney injury. Kidney Int 2013; 84: 509-520.

46) Homsi E, Janino P, Biswas SK, Mizuno S, Nakamura T, Lopes de Faria JB. Attenuation of glycerol-induced acute kidney injury by previous partial hepatectomy: role of hepatocyte growth factor/c-met axis in tubular protection. Nephron Exp Nephrol 2007; 107: e95-106.

47) Wang Z, Chen JK, Wang SW, Moeckel G, Harris RC. Importance of functional EGF receptors in recovery from acute nephrotoxic injury. J Am Soc Nephrol 2003; 14: 3147-3154.

48) Hammerman MR. Growth factors and apoptosis in acute renal injury. Curr Opin Nephrol Hypertens 1998; 7: 419-424.

49) Lin JJ, Cybulsky AV, Goodyer PR, Fine RN, Kaskel FJ. Insulin-like growth factor-1 enhances epidermal growth factor receptor activation and renal tubular cell regeneration in postischemic acute renal failure. J Lab Clin Med 1995; 125: 724-733.

50) Uhlenhuth E, Anim M, Harty JI, Howeron LW. Infundibulopelvic dysgenesis: a spectrum of obstructive renal disease. Urology 1990; 35: 334-337.

51) Kelalis PP, Malek RS. Infundibulopelvic stenosis. J Urol 1981; 125: 568-571.

52) European Polycystic kidney Disease Consortium. The polycystic kidney disease 1 gene encodes a 14 Kb transcript and lies within a duplicated region on chromosome 16. Cell 1994; 77: 881-894.

53) Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; 98: 503-517.

54) Harris PC, Thomas S, Ratcliffe PJ, Breuning MH, Coto E, Lopez-Larea C. Rapid genetic analysis of families with polycistic kidney disease 1 by means of a microsatellite marker. Lancet 1991; 338: 1484-1487.

55) Kandt RS, Haines JL, Smith M, Northrup H, Gardner RJM, Short MP, Durmars K, Roach ES, Steingold S, Wall S, Blanton SH, Flodman P, Kwiatkowski DJ, Jewell A, Weber JL, Roses AD, Pericak-Vance MA. Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. Nat Genet 1992; 2: 37-41.

56) Bernstein J. The polycystic kidney and hereditary renal adysplasia. Am J Kidney Dis 1991; 17: 495-496.

57) Somlo S, Wirth B, Germino GG, Weinstat-Saslow D, Gillespie GA, Himmelbauer H, Steevens L, Coucke P, Bachner L, Coto E, López-Larrea C, Peral B, San Millàn JL, Saris JJ, Breuning MH, Frischauf AM, Reeders ST. Fine genetic localization of the gene for autosomal dominant polycystic kidney disease (PKD 1) with respect to physically mapped markers. Genomics 1992; 13: 152-158.

58) Reeders ST, Breuning MH, Davies KE, Nicholls RD, Jarmam AP, Higgs DR, Pearson PL, Weatherall DJ. A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. Nature 1985; 317: 542-544.

59) Urbani CE, Betti R. Accessory mammary tissue associated with congenital and hereditary nephrourinary malformations. Int J Dermatol 1996; 35: 349-352.

60) Wetzel O, Hormi M, Le Normand L, Karam G, Guenel J, Auvigne J, Buzelin JM. Autosomal dominant polycystic kidney disease: urologic complications and results of kidney transplantation: 217 patients. Prog Urol 1993; 3: 252-262.

61) Gao C, Zhang L, Zhang Y, Wallace DP, Lopez-Soler RI, Higgins PJ, Zhang W. Insights into cellular and molecular basis for urinary tract infection in autosomal-dominant polycystic kidney disease. Am J Physiol Renal Physiol 2017; 313: F1077-F1083.

62) Vikrant S, Parashar A. Autosomal dominant polycystic kidney disease: Study of clinical characteristics in an Indian population. Saudi J Kidney Dis Transpl 2017; 28: 115-124.

63) Lai S, Molfino A, Russo GE, Testorio M, Galani A, Innico G, Frassetti N, Pistolesi V, Morabito S, Rossi Fanelli F. Cardiac, inflammatory and metabolic parameters: hemodialysis versus peritoneal dialysis. Cardiorenal Med 2015; 5: 20-30.

64) Lai S, Mariotti A, Coppola B, Lai C, Aceto P, Dimko M, Galani A, Innico G, Frassetti N, Mangiulli M, Cianci R. Uricemia and homocysteinemia: nontraditional risk factors in the early stages of chronic kidney disease-preliminary data. Eur Rev Med Pharmacol Sci 2014; 18: 1010-1017.

65) Zhang L, Wang F, Wang X, Liu L, Wang H. The association between plasma uric acid and renal function decline in a chinese population-based cohort. Nephrol Dial Transplant 2011; 27: 1836-1839.

66) Krumme B. Renal Doppler sonography--update in clinical nephrology. Nephron Clin Pract 2006; 103: c24-c28

67) Cianci R, Barbano B, Martina P, Gigante A, Polidori L, Lai S, Ascoli G, De Francesco I, Di Donato D, Fuiano G, Zuccala A. Nephroangiosclerosis and its pharmacological approach. Curr Vasc Pharmacol 2011; 9: 238-243.

68) Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation 2007; 115: 1285-1295

69) Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atheroscleroticrisk. Arterioscler Thromb Vasc Biol 2003; 23: 168-175

70) Lai S, Petramala L, Mastroluca D, Petraglia E, Di Gaeta A, Indino E, Panebianco V, Ciccariello M, Shahabadi HH, Galani A, Letizia C, D'Angelo AR. Hyperaldosteronism and cardiovascular risk in patients with autosomal dominant polycystic kidney disease. Medicine (Baltimore) 2016; 95: e4175.

71) Wang D, Iversen J, Wilcox CS, Strandgaard S. Endothelial dysfunction and reduced nitric oxide in resistance arteries In autosomal dominant polycystic kidney disease. Kidney Int 2003; 64: 1381-1388.

72) Kocaman O, Oflaz H, Yekeler E, Dursun M, Erdogan D, Demirel S, Alisir S, Turgut F, Mercanoglu F, Ecder T. Endothelial dysfunction and increased carotid intima-media thickness in patients with autosomal dominant polycystic kidney disease. Am J Kidney Dis 2004; 43: 854-860