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Original Paper

Early Markers of Cardiovascular Risk in Autosomal Dominant Polycystic Kidney Disease

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Key Words
Autosomal dominant polycystic kidney disease • Cardiovascular risk • Inflammation

Abstract
Background/Aims: Cardiovascular disease is the most frequent cause of morbidity and mortality in autosomal dominant polycystic kidney disease (ADPKD) patients, often before the onset of renal failure, and the pathogenetic mechanism is not yet well elucidated. The aim of the study was to identify early and noninvasive markers of cardiovascular risk in young ADPKD patients, in the early stages of disease. ***Methods:*** A total of 26 patients with ADPKD and 24 control group, matched for age and sex, were enrolled, and we have assessed inflammatory indexes, mineral metabolism, metabolic state and markers of atherosclerosis and endothelial dysfunction (carotid intima media thickness (IMT), ankle brachial index (ABI), flow mediated dilation (FMD), renal resistive index (RRI), left ventricular mass index (LVMI)) and cardiopulmonary exercise testing (CPET), maximal O_2 uptake ($V'O_2max$), and O_2 uptake at lactic acid threshold ($V'O_2@LT$). ***Results:*** The ADPKD patients compared to control group, showed a significant higher mean value of LVMI, RRI, homocysteine (Hcy), Homeostasis Model Assessment-insulin resistance (HOMA-IR), serum uric acid (SUA), Cardiac-troponinT (cTnT) and intact parathyroid hormone (iPTH) ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.007$, $p=0.019$; respectively), and a lower value of FMD and 25-hydroxyvitaminD (25-OH-VitD) ($p<0.001$, $p<0.001$) with reduced parameters of exercise tolerance, as $V'O_2max$, $V'O_2max/Kg$ and $V'O_2max$ (% predicted) ($p<0.001$, $p<0.001$, $p=0.018$; respectively), and

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metabolic response indexes ($V'O_2$@LT, $V'O_2$ @LT%, $V'O_2$@LT/Kg,) ($p<0.001$, $p=0.14$, $p<0.001$; respectively). Moreover, inflammatory indexes were significantly higher in ADPKD patients, and we found a positive correlation between HOMA-IR and C-reactive protein (CRP) ($r=0.507$, $p=0.008$), and a negative correlation between HOMA-IR and 25-OH-VitD ($r=-0.585$, $p=0.002$). ***Conclusion****:* In our study, ADPKD patients, in the early stages of disease, showed a greater insulin resistance, endothelial dysfunction, inflammation and mineral metabolism disorders, respect to control group. Moreover, these patients presented reduced tolerance to stress, and decreased anaerobic threshold to CPET. Our results indicate a major and early cardiovascular risk in ADPKD patients. Therefore early and noninvasive markers of cardiovascular risk and CPET should be carried out, in ADPKD patients, in the early stages of disease, despite the cost implication.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited renal disorder, characterized by progressive fluid-filled cyst development, and growth that lead to end stage renal disease (ESRD) in 50% of patients, by the age of 50-60 years [1-3]. The estimated prevalence is between 1:400 and 1:1000 live births [4]. About 8–10% of patients on renal replacement therapy (RRT) are affected by ADPKD [5-7]. Mutations in two genes, *PKD1* (localized on chromosome region 16p13.3 and encoding polycystin 1 [PC1]; ~85% of cases) and PKD2 (localized on chromosome region 4q21 and encoding polycystyn 2 [PC2]; ~15% of cases) [8-10], cause the disease. Mutations in PC1 and PC2, transmembranes glicoproteins that are colocalized to the primary cilium of the kidney tubular epithelial cells, cause lower intracellular levels of calcium and increased intracellular cyclic adenosine monophosphate, with aberrant cell proliferation and fluid secretion into cysts [11-13]. ADPKD is a systemic disease, that may involve different organs, showing a high phenotypic variability [14-15], and cardiovascular complications are the major cause of morbidity and mortality, with a cardiac-related death that is estimated to be 1.6- to 3.2-fold higher in these patients than in the general population [16-17]. Currently, there are no prognostic tools to identify ADPKD patients with high cardiovascular risk.

Materials and Methods

The study was approved by the Local Clinical Research Ethics Committee, with protocol number 3169/15. The study conforms to the principles outlined in the Declaration of Helsinki, and we obtained a written consent by each patient enrolled.

Study design and subjects

We performed an observational, cross-sectional study on 50 patients, 26 ADPKD patients and 24 control group matched for age and sex, at the University Hospital "Policlinico Umberto I" of Rome, Sapienza University of Rome, Italy. Patients were enrolled from July 2015 to April 2016.

Patients

A total of 26 patients affected by ADPKD (6 male; 20 female), and 24 control group (8 male; 16 female), were matched for age and sex. 21 ADPKD patients and 18 control group patients were hypertensive. The presence of secondary hypertension had previously ruled out. Both groups had good blood pressure control and antihypertensive therapies were continued in all patients included in the study (Table 1). Two patients of ADPKD group were smoker, and one patient of control group was a mildly smoker up to 8 years before.

Inclusion criteria

Patients aged >18 years with ADPKD

ADPKD was defined according to the Pei's criteria [18]. Estimated Glomerular Filtration Rate (eGFR) ≥ 80 ml/min

The eGFR was calculated with the abbreviated Chronic kidney disease-epidemiology formula (CKD-EPI), as defined by Levey et al [19].

Table 1. Patient' s characteristics. Data are show as mean ± standard deviation. Abbreviations: SBP, diurnal systolic blood pressure; DBP, diurnal diastolic blood pressure; ADPKD, autosomal dominant polycystic kidney disease; yrs, years; num, number

	ADPKD (n:26)	Control Group (n:24)	p value
SBP (mmHg)	122.6±15.2	120.8±9.9	n.s.
DBP (mmHg)	78.2±9.7	73.1±10.8	n.s.
duration of hypertension (yrs)	8.2 ± 4.1	6.7 ± 3.3	n.s.
Ace inhibitors (num)	8	6	n.s.
ARBs (num)	4	3	n.s.
Ca-antagonists (num)	7	8	n.s.
β-blockers (num)	2	1	n.s.

Exclusion criteria

We recorded the cardiovascular history and excluded patients affected by heart failure, neoplastic diseases, chronic liver disease, chronic obstructive airway disease, congenital heart disease, cerebrovascular disease, carotid artery stenosis, and acute coronary syndrome three months before of the study. We excluded also patients with diabetes, urinary abnormalities, suggestive of concomitant glomerular disease and urinary tract infection. Patients who refused to give consent and patients with missing data.

All patients performed the morpho-functional Magnetic Resonance (MR) without contrast medium, to exclude the presence of cerebral aneurysms, exclusion criterion of this study.

Laboratory measurements

Blood was sampled the morning after overnight fasting of at least 12 h, for laboratory assessment. 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), hemoglobin (Hb) (g/dL), were measured using standard automated techniques. The total white blood cell count (WBC) and the neutrophil, lymphocyte and platelet counts were recorded. The neutrophil-lymphocyte ratio (NLR), was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count (NLR < 2.8, was defined as low in our study). 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 that measures "intact" hormone (iPTH) (pg/mL) and 25-hydroxyvitaminD (25-OH-VitD) (ng/mL) was measured by radioimmunoassay. Cardiac troponin T (cTnT) (ng/mL) were measured using automated analyzer Elecsys ®2010 (Roche Elecsys 2010 chemistry analyzer, Cobas Integra 400 Plus Analyzer, Geislingen, Germany). Serum albumin (g/dL) was determined by bromcresol purple method. Insulin resistance was assessed using the homeostasis model assessment (HOMA-IR), originally described by Mathew et al [20]. Microalbuminuria 24 h (30-300 mg/24h) 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 a person's weight and height [weight (kg)/height^2 (m^2)].

Blood pressure measurements

Blood pressure (BP) measurements were made in the dominant arm, after 10 minutes of rest in the sitting position, using a standard automatic sphygmomanometer and cuffs adapted to the arm circumference [21]. The mean of the three measurements was recorded for statistical analyses.

The systolic and diastolic BP levels were taken as the points of appearance and disappearance of Korotkoff sounds, respectively. Hypertension was defined as systolic BP ≥140 mmHg or diastolic BP ≥ 90 mmHg on repeated measurements. We have determined Ankle Brachial Index (ABI), the measurement of the ratio of the systolic blood pressures in the ankle and in the arm (normal values 0.9-1) [22].

Echocardiography

All patients underwent transthoracic echocardiography with a commercially available cardiovascular

ultrasound system (Vivid E9, GE, Horten, Norway). Measurements of cardiac chambers were made according to established criteria [23-24]. LV ejection fraction by modified biplane Simpson method and mass index were estimated. Peak early (E) and late (A) diastolic velocities, deceleration time, left ventricular isovolumic relaxation time, and myocardial performance index were obtained using standard Doppler practices. Standard parasternal, apical, and subcostal views were used.

Common carotid intima-media thickness assessment (IMT)

Right (R) and left (L) carotid ultrasound was blindly performed by an experienced sonographer, who was unaware of the characteristics of the patients under examination. Participants were studied with the high-resolution B-mode ultrasound machine Toshiba Aplio XV (Toshiba AplioxV, Toshiba American Medical Systems, Inc., Tustin, CA, USA) equipped with a 5- to 12-MHz linear transducer with a 0.01-mm resolution, following a standardized vascular protocol [25]. IMT was measured at three points on the far walls of both left and right distal common carotid arteries, carotid bulb, and the proximal portion of the internal carotid arteries. The mean IMT was calculated as the average IMT on both sides. The value of IMT was considered normal when between 0.55 and 0, 9 mm [26].

Flow-mediated dilation brachial artery (FMD)

According to the method described by Celermajer and others [27], the endothelium-dependent vasodilation (FMD) of the brachial artery was assessed using a high-resolution B-mode ultrasound machine Toshiba Aplio XV (Toshiba AplioxV, Toshiba American Medical Systems, Inc., Tustin, CA, USA), equipped with a 5- to 12 MHz linear transducer with a 0.01-mm resolution, by the same blinded experienced ultrasonographer, following a standardized vascular protocol [28]. Flow-mediated vasodilatation was typically expressed as the change in post-stimulus diameter 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 AplioxV, Toshiba American Medical Systems, Inc., Tustin, CA, USA) equipped with a 3-3.5 MHz convex transducer. All measurements were made by a single, blinded, experienced ultrasonographer. Renal resistive index (RRI) values were determined with the mean of three separate measurements in the renal superior pole, interpolar regional and inferior pole on the level of the interlobular, interlobar or arcuate arteries in both kidneys. We used an anterior and an oblique approach, to detect the 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 RI value for each kidney, and then the average RI value for each patient was calculated as the mean of the RI in the left and right kidney [29]. 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 [30-31]. The intra-reader correlation coefficient for RRI was 0.97, whereas the inter-reader was 0.92.

Magnetic Resonance Imaging (MRI)

All patients underwent MR examinations at 3T magnet to assess the presence of intracranial arterial aneurysms and Total kidney volume. Imaging was performed with a 3.0-T MR unit (Verio; Siemens, Erlangen, Germany).

Respiratory function testing (Spirometry)

Spirometries were executed with the subjects in sitting position, wearing a nose clip according to the international guidelines [32-33]. Before testing each subject, the spirometer was calibrated using a certified 3-L syringe. A laboratory spirometer and Quark spirometry software (Quark PFT Suite Version 9.1a, COSMED, Pavona, Italy) were used to measure forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC).

Cardiopulmonary exercise testing (CPET)

All subjects performed an incremental exercise test up to peak work capacity on an electronically braked cycle ergometer (COSMED, Pavona, Italy), using the Quark b^2 system (Version 8.1a, COSMED, Pavona, Italy) according to guidelines [34]. All participants were continuously monitored by means of a 12-lead electrocardiograph. Blood pressure was measured every minute using a sphygmomanometer. Percentage of arterial oxygen saturation was continuously measured using a pulse oximeter. Oxygen uptake ($V'O_2$), carbon dioxide production ($V'CO_2$) and minute ventilation (V'E) were detected. CPET consisted of a steady-state resting period, then one minute of warm-up without load, followed by a stepwise protocol in which the work rate was increased in 1-minute intervals by increments of 10 Watt.The exercise test was considered maximal for a value of respiratory exchange ratio (RER) > 1.05. The test was continued until the point of symptom limitation (peak exercise). Subjects were asked to score their sense of breathlessness and muscle fatigue throughout the exercise and at peak exercise using Borg scale [35]. The Lactic Threshold (LT) was detected individually using the V-slope method [36]. Workload (W), LT, maximal oxygen consumption ($V'O_{2max}$) and HR peak values, BP for each subject were compared with those obtained in a group of healthy subjects matched for age, height, weight and gender.

Statistical analysis

Data management and analysis were performed using IBM® SPSS® Statistics 18.0 for Windows® software (IBM Corporation, New Orchard Road Armonk, New York, United States). The normality of variables was tested using the Shapiro-Wilk method for normal distributions. All continuous variables were expressed as mean ± standard deviation, categorical variables were expressed as number (percentage). The comparison of the data of patients, for all quantitative variables considered was performed using non-parametric Wilcoxon test and Student's t test. For comparing proportions was applied Chi-Square Test. Student's t-test or-Mann-Whitney U-test were performed to determine differences between groups. Binomial Test or Chi-square test was used for comparison of categorical data. Pearson's or Spearman's Correlation was used to determine in bivariate correlation the relationship and the strength of association between the variables. A probability value of $p < 0.05$ was considered to be statistically significant.

Table 2. Patient' s characteristics. Data are shown as mean ± standard deviation. Abbrevitions: BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; eGFR, estimated Glomerular Filtration Rate; Hb, Hemoglobin; NLR, Neutrophil / Lymphocyte ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein; iPTH, intact Parathormone; 25-OH-VitD, 25-hydroxyvitaminD; Hcy, homocysteine; cTnT, Cardiac troponin T; CRP, C-reactive protein

Variable	ADPKD	Control Group	p value
Age	31.7±9.1	27.8±4.5	0.067
BMI	22.9±2.8	22.6±2.0	0.665
SBP (mmHg)	122.6±15.2	120.8±9.9	0.632
DBP(mmHg)	78.2±9.7	73.1±10.8	0.090
eGFR (mL/min)	98.9±19.9	107.2±9.1	0.066
Creatinine (mg/dL)	0,81± 0,15	0.77±0.13	0.320
hATKV cm3	820.06±812.82		
Urea (mg/dL)	37.6±10.1	33.4±5.0	0.075
Uric Acid (mg/dL)	5.1±1.1	3.542±0.116	<0.001
Sodium (mEq/L)	140.4±1.8	140.2±2.3	0.654
Potassium (mEq/L)	4.3±0.3	4.1±0.3	0.073
Clalcium (mEq/L)	9.4±0.4	9.2±0.2	0.068
Posphorus (mEq/L)	3.5±0.5	3.3±0.5	0.114
Hb (g/dL)	13.2±1.4	13.0±1.2	0.665
Neutrofil (x10^3/mL)	3990.7±1305.1	4445.8±1497.4	0.257
Lymphocyte (10^3/mL)	1569.4±701.6	2520.2±516.0	<0.001
NLR	2.72±1.42	1.76±0.55	0.003
HOMA-IR	2.51±1.50	1.35±0.47	<0.001
Glycemia (mg/dL)	86.4±8.2	83.5±7.6	0.201
Total Colesterol (mg/dL)	176.3±32.2	156.3±28.5	0.025
HDL (mg/dL)	59.2±16.9	64.9±14.4	0.212
LDL (mg/dL)	98.8±8.2	76.0±9.4	<0.001
Tryglicerides (mg/dL)	85.7±34.0	73.3±28.2	0.170
iPTH (pg/mL)	48.4±23.2	35.9±10.6	0.019
(25-OH-VitD) (ng/mL)	14.0±9.5	28.3±6.2	<0.001
Fibrinogen (mg/dL)	312.0±79.4	267.9±44.6	0.020
Albumin (g/dL)	4.343±0.377	4.805±0.435	0.002
Hcy (µmol/L)	16.628±7.724	8.917±1.984	<0.001
CTnT(ng/mL)	0.014±0.019	0.003±0.004	0.007
CRP (µg/L)	7007.6±6950.1	1206.8±1122.1	0.002

Results

The study included 26 consecutive patients affected by ADPKD (6 male; 20 female), with a mean age of 31.7 ± 9.1 years, and 24 control group (8 male; 16 female), with a mean age of 27.8 ± 4.5 years. Population characteristics are shown in Table 2. There were no significant differences between the two groups regarding age, BMI, BP, eGFR (ml/min), sodium (mEq/L), potassium (mEq/L), calcium (mg/dL), phosphorus (mg/dL), glycemia (mg/dL), serum nitrogen (mg/dL), and Hb (g/dL), while we reported a significant difference in SUA (mg/dL), Hcy (µmol/L) and HOMA-IR, ($p<0.001$, $p<0.001$, $p<0.001$; respectively) (Table 2). Moreover, we found higher iPTH (pg/mL) in

ADPKD patients (mg/dL) respect to control group, although within normal values, (p=0.019)(Table 2) and lower 25-OH-VitD (ng/mL) (p<0.001) (Table 2). Inflammatory indexes, as NLR, CRP (µg/L) and fibrinogen, were significantly higher in ADPKD patients respect to control group (p=0.003, p=0.002, p=0.020; respectively) (Table 2). Total Cholesterol (mg/dL) and LDL (mg/dL) were significantly different between the two groups (p=0.025, p<0.001; respectively), while HDL (mg/dL) and triglycerides (mg/dL) did not show significant differences (p=0.212, p=0.170; respectively) (Table 2). Also albumin (g/dL) value, was significantly different between the two groups, although within normal values (p=0.002). Furthermore, we found significantly higher mean values of LVMI (g/m^2), RRI and cTnT (ng/mL) (p<0.001, p<0.001, p=0.007; respectively), and lower FMD value (p<0.001) (Table 2, Table 3), in ADPKD patients versus control group, while we did not found a significant difference in IMT and ABI values (p=0.932, p=0.495; respectively), between the two groups (Table 3). In ADPKD patients, the study correlation showed a positive correlation between HOMA-IR and CRP (r=0.507, p=0.008) (Fig. 1), and a negative correlation between HOMA-IR and 25-OH-VitD (r=-0.585, p=0.002) (Fig. 2). Also, we found significantly reduced parameters of exercise tolerance, as $V'O_{2max}$, $V'O_{2max}$/Kg and $V'O_{2max}$ (% predicted) (p<0.001, p<0.001, p=0.018; respectively) and metabolic response indexes ($V'O_2$@LT, $V'O_2$@LT%, $V'O_2$@LT/Kg,) (p<0.001, p=0.14, p<0.001; respectively) in ADPKD patients compared to control group (Table 4). Conversely, we found significantly increased the ventilatory equivalent for CO_2, $V'E/V'CO_2$@LT (p<0.001). HR peak was reduced in ADPKD patients (p<0.001), while no statistically significant difference was found in HR rest (p=0.351). The remaining parameters of the CPET did not show statistically significant differences between the two groups.

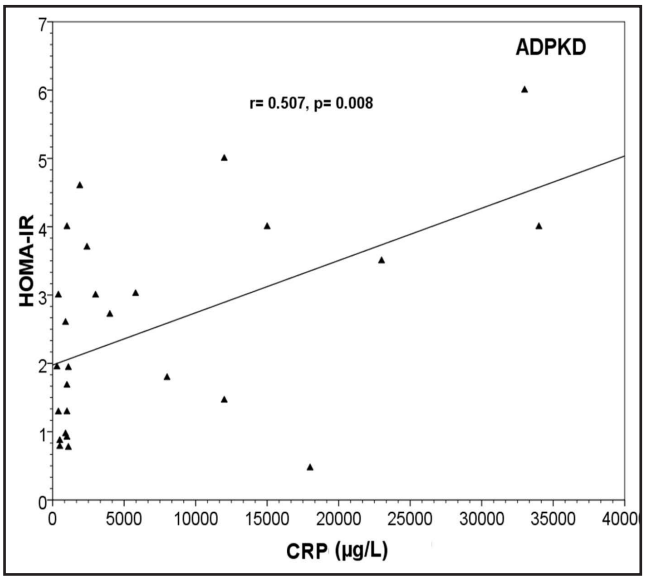

Fig. 1. Linear regression plot. Correlation between HOMA-IR and CRP (µg/L) in ADPKD patients, r=0.507, p=0.008. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; ADPKD, autosomal dominant polycystic kidney disease; CRP, C reactive protein.

Table 3. Instrumental parameters of the study participants. Data are shown as mean±standard deviation. Abbreviations: ADPKD autosomal dominant polycystic kidney disease; FMD, flow mediated dilation; cIMT, carotid intima media thickness; LVMI, left ventricular mass index; RRI, renal resistive index; ABI, ankle brachial index

Variable	ADPKD	Control Group	p value
FMD	11,23±5,66	27,46±5,29	<0,001
cIMT	0,65±0,13	0,64±0,12	0.779
LVMI	125,04±24,12	83,80±11,43	<0,001
RRI	0,64±0,057	0,55±0,03	<0,001
ABI	1.01 ± 0.06	1.00 ± 0.04	0.495

Discussion

The most important finding of our study is the early appearance of inflammatory indexes, endothelial dysfunction, atherosclerotic and metabolic markers associated with a reduction of parameters of exercise tolerance and metabolic response indexes in ADPKD patients

respect to control group. ADPKD is characterized by several cardiovascular complications, which represent the main cause of death of this disease [11]. The onset of cardiac manifestations, such as hypertension, left ventricular hypertrophy (LVH), cardiac valvular abnormalities and pericardial effusions, may precede the development of renal failure [9]. Despite the pathogenetic mechanisms are not well understood, endothelial dysfunction, inflammation, and insulin resistance, are the most commonly encountered risk factors in the pathogenesis of cardiovascular disease in ADPKD patients, also in the early stages of disease [37]. Leier *et al.* [11] hypothesized a generalized connective tissue dysfunction, with a deficiency or absence of type III collagen. Several lines of evidence, suggest a hyperactivation of renin-angiotensin-aldosterone system (RAAS), with ectopic production of renin, angiotensinogen , angiotensin-converting enzyme and angiotensin II by cystic epithelium [2, 12]. Angiotensin II seems to represent the main stimulus for the production of aldosterone, and the presence of hyperaldosteronism in ADPKD, could contribute to the development of insulin resistance and endothelial dysfunction, with impaired nitric oxide-related vasorelaxation, and progression of cardiorenal disease [15]. Recently, some authors showed an essential role of the cilia in proper development of the vascular system [16]. Insufficient expression of PC1 or PC2 could be associated with functional or structural abnormalities of the vascular system, determining a reduced release of nitric oxide with altered endothelial response to shear stress and reduced vasodilation [17]. Our data confirm that ADPKD patients, with preserved renal function, show a significant higher LVMI (Table 3), which is likely multifactorial, and may be influenced by vitamin D deficiency, hyperparathyroidism and insulin resistance [38]. In this study, we showed higher mean value of iPTH, although remaining within the normal range, and lower 25-OH-VitD levels in ADPKD

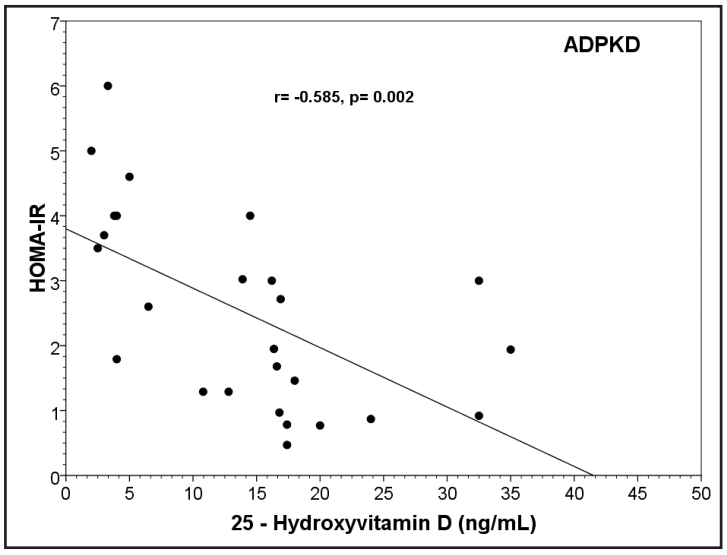

Fig. 2. Linear regression plot. Correlation between HOMA-IR and 25-OH-VitD (ng/mL) in ADPKD patients, r=-0.585, p=0.002. Abbreviations: HOMA-IR, homeostasis model assessment of insulin resistance; ADPKD, autosomal dominant polycystic kidney disease; 25-OH-VitD, 25-Hydroxyvitamin D.

Table 4. Instrumental parameters of the study participants. Data are shown as mean±standard deviation. Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; $V'O_{2max}$, maximum oxygen uptake; $V'O_{2max}$ (% predicted), maximum oxygen uptake percent of predicted; $V'O_{2max}$/Kg, maximum oxygen uptake ratio for kilogram; $V'O_2$@LT, oxygen uptake at lactic threshold; $V'O_2$@LT (% predicted), oxygen uptake at lactic threshold percent of predicted; $V'O_2$@LT/Kg, oxygen uptake at lactic threshold ratio for kilogram; $V'E/V'CO_2$@LT, ventilatory equivalent ratio for carbon dioxide at lactic threshold; HR, heart rate

Variable	ADPKD	Control Group	p value
$V'O_{2max}$ (ml/min)	1646,00±395,69	2430,20±503,08	<0,001
$V'O_{2max}$ (% predicted)	79,74±13,82	90,09±16,21	0,018
$V'O_{2max}$/Kg (ml/min/Kg)	25,04±3,36	33,62±5,03	<0,001
$V'O_2$@LT (ml/min)	906,67±163,95	1299,79±415,46	<0,001
$V'O_2$@LT (% predicted)	40,42±8,58	47,68±11,52	0,014
$V'O_2$@LT/Kg (ml/min/Kg)	13,07± 3,36	17,87±4,68	<0,001
$V'E/V'CO_2$@LT	28,24±2,94	24,61±3,37	<0,001
HR rest (bpm)	80,36±15,34	84,04±11,86	0,350
HR peak (bpm)	155,04±18,95	172,79±11,13	<0,001

patients, compared to control group. Although the involvement of Vitamin D in ADPKD is not fully elucidated, it is known that it is responsible of pleiotropic effects, by binding to the vitamin D receptor (VDRs), in non-classical targets, including cardiovascular, immune-endocrine, and nervous systems [26]. Moreover, Vitamin D deficiency reduces intestinal calcium absorption by more than 50%, and determines an increase in the levels of iPTH. An increased iPTH level may be associated with left ventricular and vascular medial smooth muscle hypertrophy. Vitamin D deficiency, and/or increased iPTH, could, at least partially, explain hypertrophy of the left ventricle and proliferation of vascular smooth muscle cells, promoting atherosclerosis and endothelial dysfunction [39-40]. Vitamin D is also involved in glycemic control, insulin secretion, and sensitivity, partially explaining the negative correlation found in our study, between 25-OH-VitD and HOMA-IR (Fig. 2). Currently, the link between polycystin function and insulin resistance, in ADPKD patients with preserved renal function, is still poorly known [41], even if recently Mao *et al*. [42] showed that polycystin proteins could regulate insulin secretion, being expressed in pancreatic islet beta cells. Other significant metabolic alterations were observed in ADPKD patients in our study, like higher Hcy and SUA respect to control group. Both of them are independent risk factors for atherosclerosis and cardiovascular disease [26], and could influence the endothelial dysfunction, through the generation of oxidative stress, promoting the development of inflammation and atherosclerosis [43]. Moreover in ADPKD patients, SUA may be considered an independent factor for renal progression [44], and associated with earlier onset of larger kidney volume, hypertension, and increased hazard for ESRD [45-46]. Chronic inflammation has been shown to be an independent predictor of cardiovascular mortality in ADPKD patients [47]. In our study, inflammatory markers as CRP, fibrinogen levels and NLR were significantly higher in patients with ADPKD compared to control group, in the early stages of disease. Moreover, CRP showed a positive correlation with HOMA-IR (Fig. 1). In recent years, NLR was introduced as a potential marker to determine inflammation in cardiac and noncardiac diseases [48]. The early metabolic and inflammatory changes that we showed, could explain the lower FMD value, and the higher mean values of LVMI and RRI, which are early markers of endothelial and cardiac dysfunction, in ADPKD patients respect to control group [49-51]. Conversely, in our study, we have not revealed a significant difference in IMT and ABI, indexes of subclinical atherosclerosis, perhaps because ADPKD patients are still in the early stages of disease. Indeed, endothelial dysfunction is an early and potentially reversible manifestation of atherosclerosis, with multifactorial etiology [52-54], and it develops in ADPKD patients, preceding the renal failure [55-56]. Atherosclerosis has been considered to play a key role in the early phases of cardiovascular disease found in ADPKD [57-59]. Cardiopulmonary exercise testing is considered the gold-standard for assessment of cardiorespiratory fitness. Measurement of maximum oxygen uptake ($V'O_{2max}$) is the most important parameter used in the evaluation of exercise tolerance. Cardiopulmonary exercise testing measures the integrated physiological responses to exercise of respiratory, cardiovascular, and skeletal muscle systems [60]. In our study, we found a statistically significant reduction of parameters of exercise tolerance as $V'O_{2max}$, $V'O_{2max}$*/Kg and* $V'O_{2max}$ (% predicted), and metabolic response indexes ($V'O_2$ *@LT,* $V'O_2$ *@LT %,* $V'O_2$ *@LT/Kg*,) (Table 4), in ADPKD patients compared to control group. The ventilatory equivalent for CO2 at lactate threshold ($V'E/V'CO_2$@LT) was significantly increased in ADPKD patients (Table 4). Impaired physical capacity in young patients with ADPKD could be explained by underlying endothelial dysfunction, which could depend on inadequate response of nitric oxide, asymmetric dimethylarginine, and BP to acute exercise [36, 61]. Several studies have identified $V'E/V'CO_2$, anaerobic threshold, and $V'O_{2max}$ as prognostic factors in cardiovascular diseases [62-63]. We found, also, a significant increase of cTnT in ADPKD patients, a sensitive and specific marker of ischemic myocardial damage, renally cleared, and widely used as a predictor of cardiovascular events [53]. Our results indicate an increase of the indexes of inflammation and endothelial dysfunction, with reduced tolerance to stress and decreased anaerobic threshold to CPET, in young ADPKD patients, with preserved renal function, in the early stages of disease, compared to control

group. Moreover CPET could allow an early assessment of maximum oxygen uptake and anaerobic threshold, and it could be used in the follow up to assess response to possible therapeutic strategies, as vitamin D supplement, hyperuricemia and hyperhomocysteinemia correction, diet and regular physical activity, that could improve the exercise tolerance and metabolic state [64].

Limitation of the study

This study was conducted in relatively small cohort of ADPKD patients; therefore should be considered as hypothesis generating data, which need to be confirmed by further clinical studies with a larger number of patients. Moreover, it is based on associations with surrogate end points, indeed it demonstrated an 'association', rather than a 'causality' relationship; the generated hypothesis thus needs further prospective follow-up studies with a larger number of patients and stronger end points to show causality.

Conclusion

Cardiovascular manifestations are common in ADPKD, and starts very early during the course of the disease. There are currently no prognostic tools to identify ADPKD patients with high cardiovascular risk [65]. The early and noninvasive evaluation of inflammatory indexes, endothelial dysfunction, atherosclerotic markers and HOMA-IR, in addiction to mineral metabolism indexes and cTnT may be necessary to improve the cardiovascular prognosis of affected patients, especially in the early stages of disease, when renal function is still preserved, despite the cost implication. Large clinical trials are needed to establish cardiovascular risk in ADPKD patients and further studies are necessary to determine if the progression of ADPKD is modified by treatment risk factors, such as vitamin D deficit, insulin resistance, hyperuricemia and hyperhomocysteinemia [66].

Disclosure Statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. The manuscript has been seen and approved by all authors. The manuscript is not under consideration for publication elsewhere.

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