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Research Article

Systemic Succinate, Hypoxia-Inducible Factor-1 Alpha, and IL-1β Gene Expression in Autosomal Dominant Polycystic Kidney Disease with and without Hypertension

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Keywords
Hypoxia-inducible factor-1 alpha · Hypertension · Polycystic kidney disease · Succinate

Abstract
Background and Objectives: Cyst pressure induces renin-angiotensin-aldosterone system activation and kidney hypoxia in autosomal dominant polycystic kidney disease (ADPKD). Lipopolysaccharide-induced Toll-like receptor activation causes metabolic disturbances that are triggered by increased succinate levels and hypoxia inducible factors, which results in inflammation via *IL-1β* activation. Since we aimed to investigate the role of both inflammation and hypoxia in the clinical course of ADPKD, via succinate levels from sera samples, *HIF-1α* gene expression from whole blood and urine samples and *IL-1β* gene expression from whole blood were measured. ***Methods:*** One hundred ADPKD patients and 100 matched healthy controls were enrolled to this cross-sectional study. Twenty-four-hour ambulatory blood pressure monitoring was conducted in all participants. Blood, serum, and urine samples were taken after 12-h fasting for the measurement of biochemical parameters and succinate levels. Whole

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blood and urine samples were used for *HIF-1α* and *IL-1β* gene expression by using quantitative real-time PCR. ***Results:*** There were significant differences in whole blood *HIF-1α*, *IL-1β* gene expression, and serum succinate levels between the ADPKD patients and the control subjects. Whole blood *HIF-1α* gene expression, *IL-1β* gene expression, and serum succinate levels were also significantly different in ADPKD patients with hypertension in comparison with normotensive ones ($p < 0.05$). Serum succinate levels and blood *IL-1β* gene expression were increased in ADPKD patients with high levels of *HIF-1α gene* expression ($p = 0.018$ and $p = 0.029$, respectively). ***Conclusions:*** Increased age, low eGFR, and *HIF-1α* and *IL-1β* gene expressions were also independently associated with hypertension in ADPKD patients. Inflammation and hypoxia are both relevant factors that might be associated with hypertension in ADPKD.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary cause of end-stage renal disease, with an estimated prevalence of 1 in 400 to 1,000 individuals. The disease is caused by mutations in either of the two genes encoding plasma membrane-spanning polycystin 1 and polycystin 2 (*PKD1* and *PKD2*, respectively) [1]. Despite identification of the genetic cause, progression of the disease has not been clarified completely. The prominent feature of ADPKD is progressive cystic kidney enlargement, which results in both renal and cardiovascular manifestations. Hypertension is a common finding that occurs in approximately 60% of patients before renal function decline and affects patient survival [2]. Although the role of renin-angiotensin-aldosterone system (RAAS) activation plays a major role in hypertension pathogenesis, other factors including sympathetic hyperactivity, endothelial dysfunction, inflammation, oxidative stress, and arterial stiffness contribute to hypertension occurrence [2, 3–5]. Moreover, variability in the ADPKD population has led researchers to investigate possible contributing factors associated with hypertension and disease progression.

The innate immune system acts as the first line of defense against invading pathogens. Toll-like receptors (TLRs), a family of receptors called pattern recognition receptors, recognize highly conserved pathogen-associated molecular patterns as well as host-derived danger signals [6]. Evidence suggests that *TLRs* have important roles in kidney diseases through the regulation of inflammatory and tissue-repair responses to infection and injury. These receptors are protective in the host defense and play a crucial role in self-perpetuating tissue damage in inflammatory and immune-mediated kidney diseases [7]. *TLR2* and *TLR4* play distinct roles in the pathogenesis of renal fibrosis; *TLR2* initiates pro-inflammatory responses, whereas *TLR4* mediates both pro-inflammatory and pro-fibrotic pathways [8].

The "Warburg effect" is an altered mitochondrial metabolism that has been deeply investigated and more recently, it is well established that succinate is a signal generated in response to the activation of *TLR4* by lipopolysaccharide (LPS), leading to *HIF-1α* activation. Activation of *TLR4* by LPS leads to a profound change in metabolism, including increased glycolysis and the pentose phosphate pathway. In addition, LPS alters the tricarboxylic acid cycle such that there is an increase in succinate. This occurs via the alteration in glutamine metabolism via both anaplerosis to α-ketoglutarate and the GABA shunt. Succinate then inhibits prolyl hydroxylases, increasing *HIF-1α* and promoting the expression of *IL-1β* and other genes [9]. However, these metabolic changes have not been studied in patients with ADPKD. The presence of inflammation without renal function decline and the possible role of *TLRs* in the clinical course of ADPKD have been shown by previous studies [10, 11].

It has been shown that *HIF-1α* mediates the metabolic responses to renal hypoxia and is associated with increased cell proliferation, angiogenesis, and apoptosis [12]. Furthermore, the effects of *HIF-1α* have become a target recently to investigate the potential role of hypoxia-induced mechanisms in various kidney diseases [13–15]. *HIF-1α* has been linked to tubulointerstitial fibrosis in hypertensive chronic kidney disease (CKD) via modifying genes encoding multiple vasoactive proteins [16]. Moreover, endothelial *HIF-1α* gene expressions in endothelial cells induce glomerular injury, hypertension, and CKD progression [14]. Hypoxia is a common problem in polycystic kidneys due to cyst expansion. Thus, the potential role of *HIF-1α* has been investigated in polycystic kidney animal models [15]. It has been shown that *HIF-1α* is highly expressed in the kidneys of PCK rats and is associated with pericystic hypervascularity [17]. Another study showed that hypoxia in renal cysts contributes to cyst growth via *HIF-1α*-dependent calcium-activated chloride secretion [18]. These results suggest that *HIF-1α* activation may be responsible for the progression of the disease. However, the relationship between hypertension and the role of *HIF-1α* gene expression in ADPKD patients is lacking. Therefore, we hypothesized that cyst pressure and local renal ischemia and inflammation might be involved in both hypertension and renal structure deterioration via hypoxia-induced local factors such as HIF-1α.

Thus, we aimed to investigate the possible role of inflammation and hypoxia in the clinical course of ADPKD, which is monitored by serum succinate levels and *HIF-1α* and *IL-1β* gene expression in urine and whole blood.

Methods

Study Population

One hundred ADPKD patients were enrolled in this study from the Turkish Society of Nephrology Polycystic Kidney Disease Working Group Registry in Kayseri, Turkey. The diagnosis of ADPKD was established on clinical grounds and family history and confirmed by the ultrasonographic criteria described by Pei et al. [19]. The study was approved by the ethics committee of Erciyes University Medical Faculty (study ID: 2016/354). After the study group was assembled, patients were asked to participate after giving informed consent. Enrolled patients were reevaluated in terms of systemic inflammation, urinary tract stones, and infection. A urinary infection was defined as an infection only if confirmed by one or more positive urinary cultures. None of the patients showed any signs of either stones or infection. Based on these, we recruited a matched control group (n = 100) from people who were admitted to our family medicine center for routine check-ups. Inclusion criteria were no known diseases and not currently taking any drugs.

The enrolled patients were reevaluated for biochemical parameters as described below. The estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20]. Blood samples were taken from the antecubital fossa vein, with subjects in a seated position and following a 20-min rest after 12 h of fasting. Glucose, creatinine, and lipid profiles were determined using standard methods.

RNA Extraction from Whole Blood and Urine Samples

Urine Samples

Ten-milliliter urine samples were centrifugated at 3,000 rpm for 10 min. The supernatant was discarded and urinary cell pellet was resuspended. Both urine and blood mRNA expression profiling was performed with total RNA extraction from whole blood by the TRIzol-based extraction method (Roche, Germany).

Blood Samples

Two-milliliter venous blood samples with ethylenediamine tetra acetic acid (EDTA) were taken from patients and the control group in order to study gene expression by quantitative real-time PCR (QRT-PCR). Total RNA extractions were obtained using TriPure Isolation Reagent (Roche, Germany) from blood samples with EDTA according to the manufacturer's instructions.

Urine and blood RNA pellets were diluted in nuclease-free water (Qiagen, Germany) to raise the amount of RNA. Biospec-Nano spectrophotometry (Shimadzu Biotech, Kyoto, Japan) was performed in order to

determine quality controls of the diluted RNA. The final RNA concentration was determined with a NanoDrop 2000 spectrophotometer. RNA samples were stored at −80 °C until used.

cDNA Isolation and QRT-PCR for HIF-1α and IL-1β Gene Expression

First-strand cDNA synthesis was performed with 1 μg of diluted RNA from each sample using a cDNA synthesis kit (High Fidelity cDNA Synthesis Kit, Roche, Germany). This procedure was conducted according to the manufacturer's protocol. cDNA was amplified for 10 min at 29 °C, for 60 min at 48 °C, and for 5 min at 85 °C. QRT-PCR reactions were performed using a Roche Light Cycler 480 for the HIF-1α, IL-1β, and ACTB (β-actin) gene. The cycling conditions were as follows: 95 °C for 10 min, followed by 45 cycles at 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 1 min. A final cooling phase had one cycle of 30 s at 40 °C. All samples were run in duplicate. The mean for each sample was calculated in order to use for statistical. The QRT-PCR data were normalized using the $2^{\wedge\wedge CT}$ derivative method.

Measurement of Succinic Acid Level

Two-milliliter venous blood samples with serum separation tube were taken from patients and control group to determine succinic acid level. Serum of patients was obtained. Succinate Calorimetric Assay Kit (BioVision, France) was used in order to get succinate level with strong absorbance at 450 nm. This procedure was conducted according to the manufacturer's instructions. Absorbance of succinic acid of each sample was measured using The GloMax-Multi Microplate Multimode Reader (Promega, USA) at 450 nm.

Ambulatory Blood Pressure Measurements

Twenty-four-hour blood pressure monitoring was performed using a Del Mar Medical Ressurometer Model P6 (Del Mar Reynolds, Irvine, CA, USA), and the results were assessed using the manufacturer's computer software. Ambulatory measurements were conducted once every 15 min from 7 a.m. until 11 p.m., and once every 30 min from 11 p.m. until 7 a.m. Evaluation was performed taking the mean values of day and night blood pressures into account. Hypertension was considered to be present if the average systolic pressure was ≥130 mm Hg and/or the average diastolic pressure was ≥80 mm Hg for a whole day or if the individual was taking antihypertensive medication.

Pulse Wave Velocity

Vascular studies were performed in a quiet, temperature-controlled room with subjects resting in a supine position. Systolic and diastolic blood pressures were measured in duplicate using a semi-automated, noninvasive oscillometric sphygmomanometer, following a 10-min rest period. Pulse wave analysis, measured in the carotid and femoral arteries was conducted using a pulse wave velocity machine (Micro Medical Pulse Trace, Rochester, UK) in accordance with the manufacturer's recommendations. Briefly, the transducers were positioned over the carotid and femoral arteries, always on the right side of the body. Pulse wave velocity was automatically calculated by measuring the time for the pulse wave to travel between the carotid and femoral arteries. All measurements were performed over 15 heart beats by a single operator blinded to the patient's grouping exposure.

Echocardiography

All participants were examined at inclusion using a Vivid 7 instruments (GE Medical Systems, Milwaukee, WI, USA), with a 2.5-MHz transducer and harmonic imaging. The echocardiographies were performed by a specialist in cardiology in the local echocardiographic laboratory and according to the recommendations of the American Society of Echocardiography. Echocardiographic examinations were conducted in the left lateral decubitus position using parasternal long-short axis and apical views. At least 3 consecutive beats in sinus rhythm were recorded, and the average values were taken. The LV end-diastolic and end-systolic dimensions (LVEDD and LVESD) and interventricular septal and posterior wall thicknesses (IVSd and LPWd) were measured from M-mode images of the left ventricle generated in the long-axis view with the cursor at the tip of the mitral valve leaflets. The LV ejection fraction was calculated using the formula: LVEF % = (LVEDV–LVESV)/LVEDV ×100. The left ventricular mass (LVM) was calculated using the formula: LVM = 0.8 × (1.04(IVSd + LVEDD + LPWd)^3 – (LVEDD)^3) + 0.6 g [21].

Endothelial Function Test

Endothelial dysfunction was assessed according to the method described by Celermajer et al. [22]. Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology

Table 1. Comparison of demographic and laboratory features between the ADPKD patients and controls

Variable	Groups		*p*
	ADPKD (*n* = 100)	control (*n* = 100)	
Demographical parameters			
Age, years	45.14±12.94	42.11±11.55	0.088
Male gender, %	48	44	0.667
Anti-hypertensive medication			
ACEI/ARB	14	–	
CCB	4	–	
Beta blocker	1	–	
Diuretic	2	–	
ACEI/ARB+CCB	4	–	
ACEI/ARB+diuretic	6	–	
BMI	26.41±5.45	25.04±4.23	0.250
Biochemical parameters			
eGFR, mL/min/1.73 m^2	81 (46–106)	101 (94–117)	<0.001
Albumin g/dL	4.35±3.84	3.94±0.37	0.592
Hemoglobin, g/L	13.93±1.7	14.22±1.57	0.444
Glucose, mg/dL	87.5 (80–96)	86 (80–90)	0.405
Proteinuria, g/day	0.43 (0.1–0.97)	0.13 (0.05–0.18)	0.018
BUN, mg/dL	16 (13–27)	13 (12–17)	0.035
Calcium, mg/dL	9.36±0.48	9.25±0.42	0.278
Phosphorus, mg/dL	3.35±0.53	3.2±0.49	0.196
Uric acid, mg/dL	5.75 (4.4–7)	4.9 (3.8–6.1)	0.011
LDL cholesterol, mg/dL	121.29±36.01	119.17±29.98	0.761
Total cholesterol, mg/dL	197.66±39.26	185.16±32.35	0.146
Physiological parameters			
Serum *HIF-1α* mRNA	1.65 (1.15–3.29)	0.61 (0.35–0.88)	<0.001
Urine *HIF-1α* mRNA	0.91 (0.38–2.50)	0.88 (0.33–1.92)	0.323
IL-1β mRNA	0.82±0.24	0.42±0.11	<0.001
Succinate, μg/μL	0.00089±0.00028	0.00054±0.00011	<0.001
Cardiovascular parameters			
Mean BP, mm Hg			
24 h	88 (84–91)	81 (78–82)	<0.001
Daytime	95 (91–98)	88 (83–90)	<0.001
Nighttime	82 (79–85)	77 (73–79)	<0.001
PWV, m/s	7.45±1.02	6.15±0.53	<0.001
FMD, %	7.26±1.06	8.98±1.22	<0.001
LVM, g	147.5±14.9	132.5±9.15	<0.001

Values are expressed as *n* (%), mean ± standard deviation, or median (1st to 3rd quartiles). BMI, body mass index; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; LDL cholesterol, low density lipoprotein cholesterol; IL-1β, interleukin-1 beta; BP, blood pressure; PWV, pulse wave velocity; FMD, flow-mediated dilatation; LVM, left ventricular mass.

Laboratories Inc., Bothell, WA, USA) with a 12-MHz probe. The subjects remained at rest in the supine position for at least 15 min before the examination started. Each subject's right arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single 2D frames. All ultrasound images were recorded on Super Video Home System (S-VHS) videotape for subsequent blinded analysis. The maximum flow-mediated vasodilation (FMD) diameters were calculated as the average of the three consecutive maximum diameter measurements after hyperemia. The FMD was then calculated as the percentage change in diameter compared with baseline resting diameter.

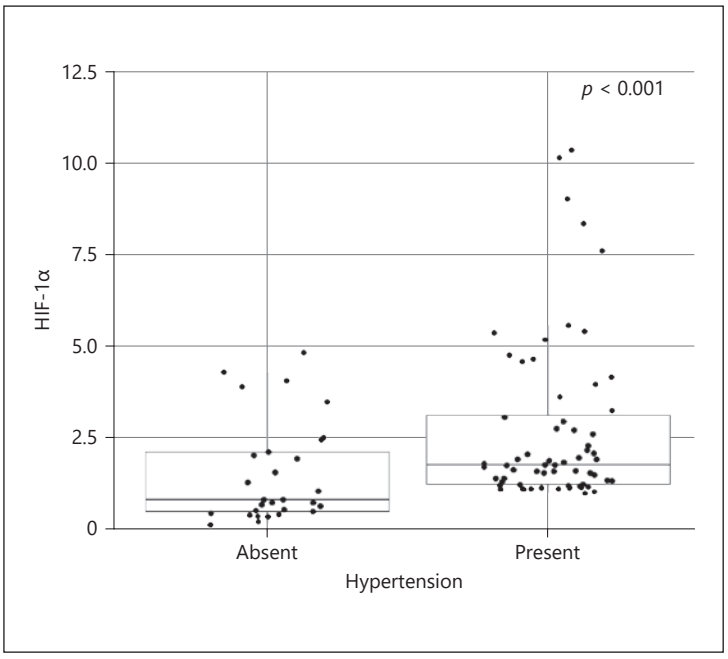

Fig. 1. The comparison of serum HIF-1α gene expression between hypertensive and normotensive ADPKD patients.

Statistical Analysis

Shapiro-Wilk test, histogram, and Q-Q plots were examined to assess the data normality. The Levene test was used to test variance homogeneity. Pearson's χ^2 analysis and Fisher's exact test were applied to compare the differences between categorical variables; independent-samples *t* test and Mann-Whitney U tests were applied to compare the differences between continuous variables. One-way analysis of covariance was performed to analyze the data by adjusting the *p* values by age. Univariate and multiple logistic regression analyses were applied to determine the risk factors of hypertension in ADPKD patients. Univariate analyses were performed for both crude and age-adjusted data. Odds ratios were calculated with 95% confidence intervals (CI). Significant variables at the 0.25 level were considered in the multiple models, and backward elimination was applied using the Wald statistic. The receiver operating characteristic (ROC) curve was also generated for *HIF-1α* gene expression as a marker in predicting hypertension in ADPKD patients. The area under the ROC curve was calculated with a 95% CI. A cutoff value was determined using the Youden index, sensitivity, specificity, and positive and negative predictive values, and the positive and negative likelihood ratios were calculated with 95% CI. Analyses were conducted using easyROC (http://www.biosoft.hacettepe.edu.tr/easyROC/) and R 3.2.0 (www.r-project.org) software. A *p* value less than 5% was considered as statistically significant.

Results

The demographical features and gene expression data from patients and controls are summarized in Table 1. Briefly, of the 100 patients, 48 were male with a mean age of 45.1 ± 12.9 years. The control group had a mean age of 42.11 ± 11.55 years, and 44% of them were male. There was significant difference in whole blood *HIF-1α* gene expression between the ADPKD patients and the control subjects ($p < 0.001$). There were also significant differences between ADPKD patients and the controls in terms of IL-1β gene expression, serum uric acid levels, serum succinate levels, mean blood pressure, pulse wave velocity, FMD, and LVM ($p < 0.001$).

Table 2. Comparison of laboratory features between ADPKD patients with and without hypertension defined as average systolic pressure ≥130 mm Hg and/or average diastolic pressure ≥80 mm Hg

Variable	Hypertension		*p*
	absent (*n* = 31)	present (*n* = 69)	
Age, years	34.21±11.25	50.02±10.48	<0.001
Male gender, %	37.9	49.2	0.879
eGFR, mL/min/1.73 m^2	110.7 (98.2–117.9)	58.1 (30–90.3)	<0.001
BMI	23.91±5.3	27.55±5.16	0.143
Albumin, g/dL	4.12±0.3	4.46±4.62	0.859
Hemoglobin, g/L	14.17±1.28	13.83±1.86	0.578
Glucose, mg/dL	81 (78–92)	90 (83–99)	0.540
Proteinuria, g/day	0.20 (0.08–0.29)	0.36 (0.11–0.97)	0.511
BUN, mg/dL	13 (12–15)	20 (14–32)	0.086
Calcium, mg/dL	9.43±0.41	9.34±0.5	0.227
Phosphorus, mg/dL	3.4±0.57	3.32±0.51	0.885
IL-1β mRNA	0.52±0.10	1.12±0.44	<0.001
Serum HIF-1α mRNA	0.85 (0.52–2.16)	1.8 (1.27–3.29)	0.010
Urine HIF-1α mRNA	0.57 (0.36–2.58)	1.2 (0.43–2.40)	0.210
Succinate, μg/μL	0.00073±0.00019	0.00094±0.00037	0.031
Mean BP, mm Hg			
24 h	83 (81–86)	89 (87–94)	<0.001
Daytime	89 (87–92)	96 (93–100)	<0.001
Nighttime	78 (76–80)	84 (81–89)	<0.001
Uric acid, mg/dL	4.5 (3.9–5.6)	6.4 (5.2–7.8)	0.005
LDL cholesterol, mg/dL	115.25±37.87	124.08±35.08	0.955
Total cholesterol, mg/dL	188.09±43.66	202.07±36.59	0.776
PWV, m/s	6.48±0.97	8.45±1.22	<0.001
FMD, %	8.21±1.26	6.32±0.93	<0.001
LVM, g	138.5±11.5	156.0±16.7	<0.001

Values are expressed as *n* (%), mean ± standard deviation, or median (1st to 3rd quartiles). eGFR, estimated glomerular filtration rate; BMI, body mass index; BUN, blood urea nitrogen; IL-1β, interleukin-1 beta; BP, blood pressure; LDL cholesterol, low density lipoprotein cholesterol; PWV, pulse wave velocity; FMD, flow-mediated dilatation; LVM, left ventricular mass.

Additionally, while there was significant difference between patients with hypertension and without hypertension in terms of serum *HIF-1α* gene expression (1.8 [1.27–3.29] vs. 0.85 [0.52–2.16], $p < 0.05$, respectively] (Fig. 1; Table 2). Serum uric acid levels and serum succinate levels were found to be significantly increased in the hypertensive group ($p = 0.005$ and $p < 0.001$, respectively) (Table 2).

Comparison of laboratory and demographical features according to tertiles of *HIF-1α* gene expression levels in ADPKD patients is summarized in Table 3. While blood pressure, proteinuria, and serum succinate levels significantly increased from low to high levels of *HIF-1α* gene expression, FMD significantly decreased inversely.

A one-unit increase in serum *HIF-1α* gene expression led to a 1.73-fold hypertension risk increase in multiple logistic regression analysis ($p < 0.05$) (Table 4). Additionally, age, *IL-1β* gene expression, and eGFR were found to be independent risk factors in identifying hypertension in ADPKD patients.

Finally, ROC analysis for *HIF-1α* gene expression values in predicting hypertension in ADPKD patients is given in Figure 2. Area under ROC curve was found to be 0.73 (0.60–0.86). A 96.9% sensitivity and 58.6% specificity was obtained for the cutoff value 1.14.

Table 3. Comparison of laboratory and demographical features according to tertiles of blood *HIF-1α* mRNA levels in ADPKD patients

Variable	Serum *HIF-1α* mRNA			*p*
	low expression (<0.52) (*n* = 33)	moderate expression (0.52–1.0) (*n* = 34)	high expression (>1.0) (*n* = 33)	
Age, years	47.13±11.41	45.34±13.68	42.94±13.66	0.445
Male gender, %	13 (41.9)	14 (43.8)	16 (51.6)	0.718
Mean BP, mm Hg				
24 h	85 (81–88)[a]	88 (85–90)[b]	93 (88–100)[c]	<0.001
Daytime	91 (88–93)[a]	94 (92–96)[b]	100 (94–107)[c]	<0.001
Nighttime	80 (76–82)[a]	82 (79.5–85)[b]	87 (81–95)[c]	<0.001
eGFR, mL/min/1.73 m^2	72.5 (48–108)	91 (41–111)	73.5 (26–95.5)	0.255
BMI	27.76±6.08	26.06±4.84	25.23±5.11	0.184
Albumin, g/dL	3.92±0.50	4.1±0.55	4.2±0.37	0.387
Hemoglobin, g/L	13.8±1.5	13.4±1.8	12.04±1.9	0.920
Glucose, mg/dL	86 (79–96)	91 (85–98)	83 (78–96)	0.108
Proteinuria, g/day	0.14 (0.09–0.21)[a]	0.16 (0.10–0.25)[a, b]	0.34 (0.11–0.74)[b]	0.038
BUN, mg/dL	17 (13–26)	16 (12–28)	18 (13–30)	0.465
Calcium, mg/dL	9.39±0.43	9.32±0.53	9.38±0.47	0.842
Phosphorus, mg/dL	3.22±0.58	3.39±0.49	3.53±0.50	0.259
IL-1β mRNA	0.52±0.11	0.79±0.19	1.09±0.29	0.018
Succinate, μg/μL	0.00077±0.00022	0.00084±0.00034	0.00104±0.00041	0.029
PWV, m/s	7.26±0.94	7.35±0.91	7.75±1.30	0.138
FMD, %	7.27±1.11[a, b]	7.61±0.90[a]	6.88±1.06[b]	0.021
LVM, g	144.87±13.39	147.07±15.79	149.94±16.85	0.543
Uric acid, mg/dL	5.9 (4.6–6.6)	5.55 (4–7.1)	5.9 (4.7–7.9)	0.587
LDL cholesterol, mg/dL	128.6±33.8	118.6±30.6	116.7±42.8	0.396
Total cholesterol, mg/dL	207.2±38.8	193±33.6	192±44.4	0.270

p: significance value adjusted by age. Values are expressed as *n* (%), mean ± standard deviation, or median (1st to 3rd quartiles). BP, blood pressure; eGFR, estimated glomerular filtration rate; BMI, body mass index; BUN, blood urea nitrogen; IL-1β, interleukin-1 beta; PWV, pulse wave velocity; FMD, flow-mediated dilatation; LVM, left ventricular mass; LDL cholesterol, low density lipoprotein cholesterol. [a, b, c] Different superscripts in the same row indicate statistically significant difference among groups ($p < 0.05$).

Table 4. Univariate and multiple logistic regression models predicting hypertension in 100 ADPKD patients

Variable	Univariate Crude OR (95% CI)	Multiple OR (95% CI)
Age, years	1.14 (1.08–1.21)	1.10 (1.02–1.19)
Male gender	ns	–
Serum *HIF-1α* mRNA	1.60 (1.09–2.36)	1.73 (0.99–3.22)
Urine *HIF-1α* mRNA	ns	–
eGFR, mL/min/1.73 m^2	0.93 (0.90–0.96)	1.03 (0.93–1.14)
Calcium, mg/dL	ns	–
IL-1β mRNA	1.11 (1.08–1.18)	1.07 (1.06–1.13)
Phosphorus, mg/dL	ns	–
Albumin, g/dL	ns	–
Uric acid, mg/dL	ns	–
LDL cholesterol, mg/dL	ns	–
Total cholesterol, mg/dL	ns	–
Glucose, mg/dL	ns	–
Proteinuria, g/day	ns	–

Adjusted by age. OR are calculated for a 10-unit increase in related variables.

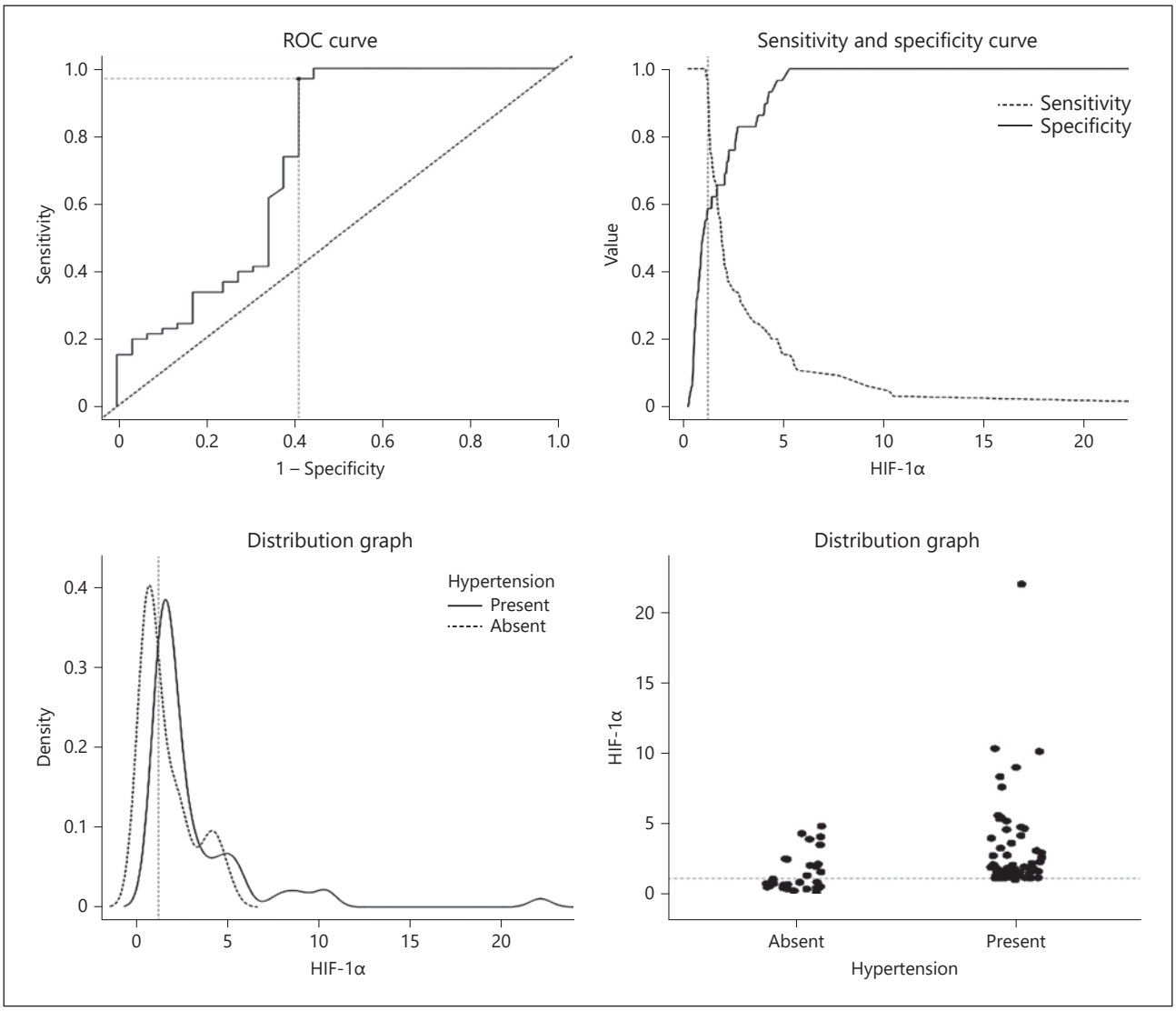

Fig. 2. ROC analysis for *HIF-1α* gene expression values in predicting hypertension in ADPKD patients.

Discussion

The present study was undertaken to explore the further potential role of serum succinate levels and whole blood and urine *IL-1β* and *HIF-1α* gene expression in the clinical course of ADPKD patients. This study proposes three major findings in patients with ADPKD. First, whole blood *HIF-1α* gene expression and *IL-1β* gene expressions are significantly increased in ADPKD patients compared to healthy controls. Secondly, blood *HIF-1α* and *IL-1β* gene expressions are increased in hypertensive ADPKD patients compared to normotensive ones. Thirdly, increased blood *HIF-1α and IL-1β* gene expressions, age, and decline of eGFR independently predict hypertension.

Early development of hypertension is one of the important clinical problems in ADPKD patients and often leads to diagnosis of the disease [1]. Hypertension occurs at a much earlier age in patients with ADPKD (median age 32 years for males, 34 years for females) compared to patients with essential hypertension (median age 45 years) [23]. Hypertension is also asso-

ciated with rapid progression to end-stage renal disease and increased risk of cardiovascular disease [24, 25]. Hypertensive ADPKD patients have larger kidneys compared to their normotensive counterparts [26]. Cystic expansion and the pressure of cysts on intact nephrons induce RAAS activation, which is mainly attributed to hypertension occurrence [2]. However, the widely accepted mechanism is RAAS activation due to possible local renal ischemia. Other factors including sympathetic hyperactivity, endothelial dysfunction, oxidative stress, intrarenal renin angiotensin system activation, and inflammation contribute to hypertension pathogenesis [3–5, 27]. Hypertension remains a potentially treatable variable in ADPKD patients. Furthermore, authors have focused on the two trigger pathogenetic mechanisms in the ADPKD population, cystogenesis and hypertension, due to their pivotal role in the progression of the disease.

The hypothesis that hypoxia is a common mediator in kidney diseases was first reported two decades ago [28]. Concurrent studies have implicated that hypoxia promotes fibrosis in all types of CKD [29–31]. More recently, Luo et al. [14] showed that the stimulation of HIF-1α gene expression in endothelial cells is deleterious in terms of developing kidney injury, hypertension, and progression of CKD. ADPKD, the most common inherited cause of CKD, is strongly linked to renal hypoxia by cystic expansion of the kidneys. Bernhardt et al. [17] firstly investigated the relationship between HIFs in PKD rats and human kidney specimens with ADPKD. They showed that HIF accumulation is associated with pericystic hypervascularity and they found histological expression of HIF-1α in the kidney specimen of ADPKD individuals. In another study, Belibi et al. [15] studied HIF-1α expression and autophagy in PKD rats. They showed that HIF-1α is highly expressed in the late stages of PKD and correlated with autophagy in a similar pattern. Lastly, Buchholz et al. [18] showed the relationship between HIF-1α and cyst expansion. The intriguing results of the study showed that HIF-1α induces calcium-activated chloride secretion in cysts and this may contribute to cyst growth. The results of the above studies showed the role of HIF-1α in PKD via different pathogenetic mechanisms. More recently, Kraus et al. [32] showed that HIF-1α was associated with the acceleration of cyst growth in mouse models of PKD. They also concluded that HIF-1α may become a therapeutic target in ADPKD patients. However, the data about clinical studies lack the ability to show the potential role of HIF-1α in the clinical course of ADPKD. Thus, we investigated the role of blood and urine HIF-1α gene expression in hypertensive and normotensive ADPKD patients compared with healthy controls. We showed for the first time in a clinical study that blood HIF-1α mRNA gene expression independently predicts hypertension in ADPKD patients.

We have recently studied the possible role of TLR gene expression in the clinical course of patients with ADPKD and we showed that both TLR2 and TLR4 gene expressions were associated with rapid disease progression [10]. Furthermore, recent advances diverted the authors to investigate the possible interaction between inflammation via immune system activation and hypoxia, which may result in a fibrosis process.

Activation of TLRs, notably TLR4, leads to a switch from oxidative phosphorylation to glycolysis in immune cells. Macrophages are activated by the gram-negative bacterial product and LPS, switching their metabolism from oxidative phosphorylation to glycolysis [33, 34]. Tannahill et al. [35] have reported that LPS increased the tricarboxylic acid cycle intermediate succinate. LPS-induced succinate also stabilized HIF-1α. They found that LPS-induced IL-1β protein expression was dramatically increased under hypoxia and concluded that succinate is therefore identified as a metabolite in immune signaling, which causes enhanced IL-1β production during inflammation. These findings pointed out that inflammation leads to glycolysis and increased succinate might be related to HIF-1α and IL-1β activation. In this study, we also demonstrated that patients with ADPKD and hypertension have higher levels of both succinate and IL-1β gene expression in comparison with normotensive ones. Our study results showed the potential association between inflammation and hypoxia.

In conclusion, the present study showed that decreased eGFR, increased age, and most importantly, increased serum succinate levels and total blood *HIF-1α* and *IL-1β* gene expressions are associated with hypertension in ADPKD. Therefore, drug development and potential therapeutic approaches targeting inflammation and hypoxia via *HIF-1α* by modulation of epigenetic status in ADPKD may become an attractive area of research.

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Statement of Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Disclosure Statement

All authors declare that there is no conflict of interest.

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Author Contributions

I.K., E.E., and S.T. conceived of the study and designed the protocol, which was implemented by I.K., J.A., E.F.S., and E.E. These authors also recruited the participants. B.T., M.H.S., O.O., and Y.O. helped with the study design. S.T. and E.F.S. set up and validated the inflammatory markers assay and helped to interpret the results. E.T., E.M., and K.K.B. performed the tests for determining mRNA expressions and succinate measurements. I.K. and E.E. coordinated the study. I.K. and I.U. collected the data of the study. G.Z. performed the statistical studies. I.K. and E.E. wrote the paper. J.A. reviewed the draft and provided expertise for revision. All authors participated in data analysis and interpretation and also read and approved the final manuscript.

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