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Research Article

Angiogenic Factors Predict Progression in Young Patients with Autosomal Dominant Polycystic Kidney Disease

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Abstract

Background: There are no robust circulating biomarkers that predict the rate of decline in renal function in patients with autosomal dominant polycystic kidney disease (ADPKD). In cross- sectional analysis vascular endothelial growth factor (VEGF) has been associated with kidney disease severity and increased left ventricular mass index in ADPKD. Thus, we hypothesized that levels of VEGF and related angiogenic factors may predict the rate of renal structural and functional disease progression, and LVMI increase in ADPKD.

Methods: 91 participants aged 8 - 22 years with normal renal function from the ADPKD children's statin therapy clinical trial were evaluated. Exposure variables included serum VEGF, secreted protein acidic and rich in cysteine (SPARC), thrombospondin-1 (TSP1) and soluble fms-like tyrosine kinase 1 (sFlt1). Outcome variables included change in height corrected total kidney volume (HtTKV), creatinine clearance, urine albumin excretion (UAE) and LVMI determined over 3 years.

Results: Higher LN urine VEGF at baseline predicted a greater decrement in UAE after 3 years, independently of statin treatment ($P < 0.05$). A higher baseline level of serum VEGF predicted a greater decrease in creatinine clearance, and increase in LVMI independent of intervention ($P < 0.05$). Higher baseline levels of serum SPARC and TSP-1 likewise predicted a decrease in creatinine clearance independent of treatment ($P < 0.05$).

Conclusion: The results suggest that urine VEGF excretion may be an independent predictor of albuminuria in young ADPKD patients. Likewise, higher serum levels of VEGF, SPARC and TSP-1 predict a decrease in renal function thus these factors may represent new biomarkers of disease progression worth exploring in future large populations.

Keywords: *Autosomal Dominant Polycystic Kidney Disease; Vascular Endothelial Growth Factor; Secreted Protein Acidic and Rich in Cysteine; Thrombospondin-1; Urinary Albumin Excretion; Creatinine Clearance*

Abbreviations

ADPKD: Autosomal Dominant Polycystic Kidney Disease; VEGF: Vascular Endothelial Growth Factor; SPARC: Secreted Protein Acidic and Rich in Cysteine; TSP-1: Thrombospondin-1; UAE: Urinary Albumin Excretion

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common potentially lethal genetic disease that affects 1 in 1000 people [1]. It accounts for 5 - 10% of end-stage renal disease (ESRD) cases in the US with half of affected patients reaching ESRD by age

60 [2]. ADPKD is characterized by development of multiple cysts which originate from the renal tubules [3]. Cyst growth results in enlargement of the kidneys with loss of normal renal parenchyma and eventual loss of renal function. The pathogenesis of the disease is still unclear and there remains no effective approved treatment in the US to date. The involvement of 2 different causative genes, *PKD1* and *PKD2* [3], in addition to the effects of modifier genes adds complexity to prediction of disease course in an individual patient. Despite the fact that interventions are likely to be most effective when initiated early in disease before irreparable renal damage has occurred, there is currently no accurate method to predict prognosis in children where renal function remains apparently normal, underlining the need for sensitive predictive biomarkers that may be implemented in early disease.

Although ADPKD is a non-neoplastic lesion, cyst growth resembles the growth of a benign tumor. ADPKD kidneys are characterized by a variety of defects, including dysregulated cystic tubular cell proliferation, apoptosis and angiogenesis [4-7]. Previous studies have revealed abnormalities in the renal vasculature in polycystic kidneys based on angiography [4-9], suggesting that angiogenesis may play a role in progression. Furthermore, the key mediator of angiogenesis, vascular endothelial growth factor (VEGF) [10] has been identified in renal and hepatic cysts of ADPKD patients [11,12]. Moreover, in cross-sectional analysis we have previously shown that serum VEGF level correlated with both renal structure and function in young patients with ADPKD [13]. VEGF has also been shown to play a role in cardiac development and disease including cardiac hypertrophy [14,15]. However, so far no robust circulating biomarkers that predict the rate of disease progression in ADPKD have been identified. Thus, we hypothesized that the baseline level of VEGF and associated regulator levels [thrombospondin 1 (TSP1), (SPARC) and soluble fms-like tyrosine kinase-1 (sFlt1) [16-19] may predict the rate of ADPKD progression. In order to test this hypothesis, we utilized samples from the recently published study showing that pravastatin treatment significantly slowed the progression of structural kidney disease in children and young adults with ADPKD [20].

Materials and Methods

Study patient population

The study population included ninety-one ADPKD patients aged 8 - 22 years who participated in a clinical trial to evaluate the effect of statin therapy on disease progression in ADPKD (NCT00456365). The study was approved by Colorado Multiple Institutional Review Board (COMIRB). All participants (or parents as appropriate) signed informed consent. Diagnosis of ADPKD was based on results of imaging [21]. All subjects completed both the baseline and 36-month study visits. Patients were evaluated during a 2-day in-patient stay at the Children's Hospital of Colorado which included magnetic resonance assessment of kidney volume and cardiac left ventricular mass [20]. All patients received the angiotensin converting enzyme inhibitor Lisinopril (2.5 mg/d to the maximum of 0.5 mg/kg based the patients' blood pressure) throughout the study and were randomized to pravastatin treatment (20 – 40 mg daily according to age) or placebo for 36 months. Details of the study have been reported previously [20]. 91 patients who completed both baseline and 36 month visits with available corresponding 24-hour urine samples were included in the analyses. At baseline 21 female and 6 male subjects had albuminuria (urine albumin excretion 20 - 200 μg/min) and 4 male subjects had clinical albuminuria (albumin excretion > 200 μg/min).

Serum and urine collection and chemistries

Sera or urine samples were not available for some patients and only those patients with samples available at both visits were included as indicated (Table 1). Blood was collected and serum separated by centrifugation. Serum was stored at -80˚C prior to assay. A total of 2, 24-hour urine collections were collected on ice during each visit. Urine volume was measured and urine aliquots stored at -80˚C prior to assay. Serum and urine creatinine level and urine albumin were measured in the hospital clinical laboratory by standard methods.

VEGF, sFlt1, SPARC and TSP1 levels in urine and serum

In the current study serum and urine VEGF excretion were the main exposure variables. Secondary exposure variables were the known regulators of VEGF including serum SPARC, TSP-1 and sFlt-1. VEGF levels were measured using Quantikine enzyme-linked immunosorbent (ELISA) kits from R&D system (Minneapolis, MN). The intra- and inter-assay coefficient of variability (CV%) for VEGF assay

was 6.7 and 8.8%, respectively, with lower limit of detection, 9 pg/ml. This assay determines the level of VEGF 165 only. Serum levels of sFLt1, SPARC and TSP1 were measured by ELISA assay kits from R & D Systems. The respective assay sensitivity and precision was as follows; sFlt1 lower detection limit 13.3 pg/ml, Intra-assay and inter assay CV% 3.8 and 9.8%, SPARC lower detection limit 0.27 ng/ml, Intra-assay and inter assay CV% 2.5 and 8.5% and TSP1 lower detection limit 0.94 ng/ml, Intra-assay and inter assay CV% 6.7 and 6.2%.

Group						
	Pravastatin			Placebo		
	N	Mean	S.D.	N	Mean	S.D.
Age (year)	49	15.71	3.96	42	15.45	3.72
Gender (Male) %	20	40.8		16	38.1	
Ln (Serum VEGF (pg/ml)	44	4.78	0.08	41	4.41	0.76
(Serum sFlt1 (pg/ml))	44	95.60	21.02	40	106.95	26.53
Ln (SPARC (ng/ml))	44	6.50	0.51	40	6.46	0.56
Ln (TSP-1(ng/ml))	44	1.59	0.90	40	1.58	1.04
Ln (Urine VEGF mmol/Cr)	41	0.90	1.09	33	1.32	0.70
Ln (24h Urine VEGF(ng))	43	3.30	1.11	34	3.54	0.69
Delta [Ln(TKV (ml))]	46	0.19	0.13	41	0.25	0.15
Delta [Ln albuminuria (µg/min)]	47	0.07	0.74	41	0.02	0.95
Delta (LVMI (g/m^2))	47	4.63	13.37	40	5.44	12.43
Delta (Creatinine Clearance ml min/1.73m^2)	47	-10.72	23.14	42	-13.32	30.68

Table 1: *Baseline characteristics of the patients.*

Outcome variables

Outcome variables included changes in total kidney volume (TKV), creatinine clearance, albumin excretion and LVMI determined over 3 years. Measurements of these variables were mentioned in detail in the original study publication [20].

Statistical analysis

All variables were checked for normal distribution. A natural log transformation was performed on all variables with a skewed distribution and the transformed variables were used in all analyses. Proportions were calculated for categorical variables and mean and standard deviation (SD) were calculated for continuous variables in descriptive analyses. Linear regression models were employed to examine the association between a continuous outcome and predictors with adjustment for covariates (age, sex, treatment group). While changes from baseline in outcome measures were used as the outcome in linear regression to evaluate the effect of angiogenic factors, a binary outcome defined as a 20% or greater change from baseline to the end of study (yes/no) was also analyzed. The logistic regression model was employed to evaluate the effect of angiogenic factors on the binary outcome. In addition, a composite binary outcome defined as yes if any one individual outcome achieved a 20% or greater change over the course of the study and the logistic model applied. Due to limited sample or incomplete/missing urine collections the exposure and outcome variables were not available in all 91 patients at baseline or 3-year visit (Table 1). Thus, a separate analysis was performed using multiple imputation with the Markov Chain Monte Carlo (MCMC) method to include all the missing values. All analyses were performed by using SAS version 9.4, (Carey, N. Carolina).

Results and Discussion

Results

Baseline characteristics of the patients

Overall 91 patients completed baseline and at 36 months visits with 49 patients in pravastatin treatment and 42 patients in placebo

group completing the study. At baseline the mean patient age was 15.4 ± 3.8 years and the study group comprised 36 males and 49 female participants (Table 1). All subjects had normal renal function based on creatinine clearance. At baseline 21 female and 6 male subjects had albuminuria (urine albumin excretion 20 - 200 µg/min) and 4 male subjects had clinical albuminuria (albumin excretion > 200 µg/min). Complete baseline characteristics are depicted in table 1.

Angiogenic factor effect on urine albumin excretion

Higher baseline total daily natural log (LN) 24h urinary VEGF excretion was significantly related to a decrease in urinary albumin excretion over the course of the study. This relationship was independent of statin treatment (Figure 1 and Table 2). There were no significant relationships between baseline serum LN VEGF, LN SPARC, sFlt1, LN TSP1 levels and change in LN urine albumin excretion. Baseline LN 24h urine VEGF excretion was not related to change in creatinine clearance over time and there was also no relationship between serum VEGF and urine VEGF level.

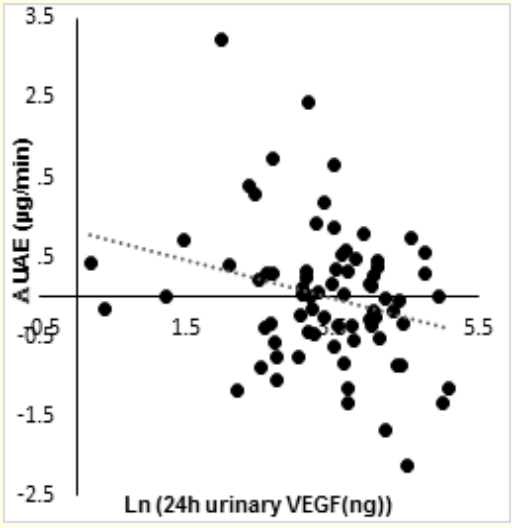

Figure 1: Scatter plot of baseline Ln (24h urinary VEGF) and change in albuminuria over 3 years*. The plot depicts the relationship between change of urine albumin excretion (UAE) (µg/min) over 3 years and the baseline Ln (24h urinary VEGF (ng)) corrected for pravastatin treatment.*

Angiogenic factor levels and creatinine clearance

Higher LN serum VEGF levels at baseline predicted a significantly greater decrement in creatinine clearance over the course of the study (Table 2) and this association was independent of statin treatment. Similarly, higher serum levels of LN SPARC and LN TSP1 at baseline predicted a significantly greater decrement in creatinine clearance over three years (Figure 2). This relationship was independent of statin treatment. Interestingly, combination of LN VEGF, LN SPARC and LN TSP1 into a model did not increase the association of the combined predictors for creatinine clearance. This may be explained by the strong inter-relationship between all 3 of these factors. The Pearson correlation coefficients were 0.39 between LN (serum VEGF) and LN (SPARC); 0.42 between LN (serum VEGF and LN (TSP-1) and 0.71 between LN (SPARC) and LN (TSP-1) respectively. All p-values were < 0.001.

Exposure Variables		
Independent Variables	**Estimate (95% Confidence Interval)**	**P-value**
	Delta (albuminuria)	
Serum sFlt-1(pg/ml)	0.0013 (-0.007, 0.0095)	0.74
Ln (24h urinary VEGF(ng))	-0.26 (-0.48, -0.05)	0.02
Ln (Serum VEGF(pg/ml))	-0.042 (-0.29, 0.20)	0.73
Ln (Serum SPARC(ng/ml))	0.023 (-0.34, 0.39)	0.90
Ln (Serum TSP-1(ng/ml))	0.014 (-0.19, 0.21)	0.89
	Delta (creatinine clearance)	
Serum sFlt-1(pg/ml)	0.20 (-0.05, 0.46)	0.11
Ln (24h urinary VEGF(ng))	-1.80 (-8.18, 4.59)	0.58
Ln (Serum VEGF(pg/ml))	-7.82 (-15.44, -0.21)	0.04
Ln (Serum SPARC(ng/ml))	-12.79 (-23.91, -1.66)	0.02
Ln (Serum TSP-1(ng/ml))	-7.07 (-13.19, -0.96)	0.02

Table 2: *Relationship between serum sFlt-1, urinary VEGF, serum VEGF, serum SPARC and serum TSP-1 with renal functional parameters including urinary albuminuria and creatinine clearance (CrCl) corrected for pravastatin treatment.*

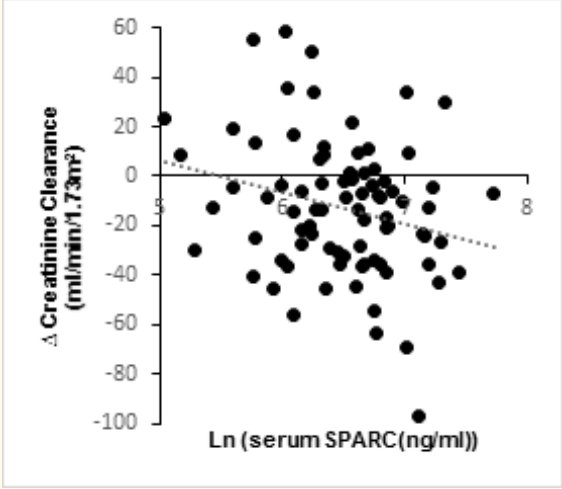

Figure 2: Scatter plot of baseline Ln (serum SPARC) and change in creatinine clearance over 3 years. *The plot depicts the relationship between change in creatinine clearance (ml/min/1.73m^2) over 3 years and the baseline Ln (serum SPARC (ng/ml)) corrected for pravastatin treatment.*

Angiogenic factor levels and height corrected TKV (TKV_Ht)

There were no significant relationships between the baseline levels of serum or urine LN VEGF, LN SPARC, sFlt1 or LN TSP1 and TKV_Ht after correction for statin therapy (Table 3).

Exposure Variables		
Independent Variables	**Estimate (95% Confidence Interval)**	**P-value**
	Delta (Ln TKV)	
Serum sFlt-1(pg/ml)	0.00008 (-0.001, 0.001)	0.90
Ln (24h urinary VEGF(ng))	0.023 (-0.011, 0.057)	0.18
Ln (Serum VEGF(pg/ml))	-0.013 (-0.051, 0.026)	0.51
Ln (Serum SPARC(ng/ml))	-0.0048 (-0.063, 0.053)	0.87
Ln (Serum TSP-1(ng/ml))	-0.024 (-0.056, 0.007)	0.13
	Delta (LVMI)	
Serum sFlt-1(pg/ml)	0.043 (-0.086, 0.17)	0.51
Ln (24h urinary VEGF(ng))	0.92 (-2.26, 4.10)	0.56
Ln (Serum VEGF(pg/ml))	3.66 (0.047, 7.27)	0.047
Ln (Serum SPARC(ng/ml))	1.56 (-3.99, 7.10)	0.58
Ln (Serum TSP-1(ng/ml))	1.47 (-1.65, 4.59)	0.35

Table 3: *Relationship between serum sFlt-1, urinary VEGF, serum VEGF, serum SPARC and serum TSP-1 with renal and cardiac structural parameters including TKV and LVMI corrected for pravastatin treatment.*

Angiogenic factor levels and LVMI

Baseline log transformed serum VEGF alone predicted an increase in LVMI over three years (Table 3, Figure 3). The relationship was independent statin treatment. There were no relationships between any of the other angiogenic factors and change in LVMI over 3 years.

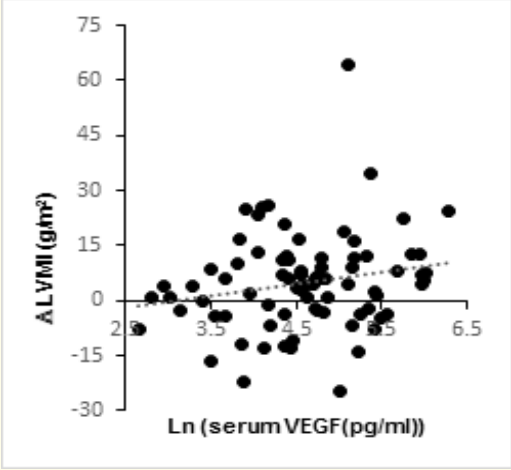

Figure 3: Scatter plot of baseline Ln (serum VEGF) and change in LVMI over 3 years. *The plot depicts the relationship between change in left ventricular mass index (LVMI) over 3 years and the baseline Ln (serum VEGF (pg/ml) corrected for pravastatin treatment.*

Effect of statin treatment on angiogenic growth factor levels

Statin therapy had no significant effect on the levels of serum or urine VEGF, or serum SPARC, sFlt1 or TSP1 levels alone or after correction for baseline level, sex and age at visit when measured after 3 years of treatment.

Association of angiogenic factors and a ≥ 20% Change in HtTKV, UAE and LVMI

We examined the association between angiogenic factors and a ≥ 20% change in Ht-TKV, UAE or LVMI as well as a composite of ≥ 20% change in either Ht-TKV, UAE or LVMI (the primary outcome of the original study) [20]. No association was found between the angiogenic factors and any of the binary outcomes.

Analyses using multiple imputation with the Markov Chain Monte Carlo (MCMC) method

When all 91 patients' data were included in the analyses using multiple imputation with the MCMC method, the results were similar to those without imputation.

Discussion

In the present study, we demonstrate a relationship between VEGF and the related factors SPARC and TSP1with the rate of renal disease progression determined over 3 years in children and young adults with ADPKD. Increased serum levels of LN serum VEGF, LN SPARC or LN TSP1 predicted a greater decrease in creatinine clearance over the study period. The serum level of each of these factors was interrelated and inclusion of all these factors into a model did not improve the strength of the predictor. In contrast increased total urinary excretion of VEGF (LN urine VEGF ng/24h) correlated with a greater decrement in urine albumin excretion over the course of the study. Higher serum LN VEGF levels at baseline predicted a greater increase in LVMI over three years.

While statin treatment decreased the rate of increase in total kidney volume over the course of the study [20], statin treatment had no significant effect on VEGF, TSP1 or SPARC levels. This suggests that the beneficial action of statin on slowing the increase in kidney growth is independent of VEGF. There have been conflicting results regarding the effect of statin on VEGF. A recent meta-analysis of human studies showed that statin treatment significantly reduced plasma VEGF concentrations [22]. This observation was only true when the duration of the treatment was ≥ 4 weeks, with use of a lipophilic statin, and a LDL-lowering effect ≥ 50 mg/dl in a population with cardiovascular disease. Indeed, statins have been shown to differentially regulate angiogenesis and VEGF in endothelial and vascular smooth muscle cells [23]. The action was dependent on cell type as well as the treatment concentration of statin. Pravastatin used in our study, is hydrophilic and the study population were young ADPKD patients without hyperlipidemia. These differences might account for the absence of statin effect on VEGF levels observed in the current study.

In the current study, there was no correlation between serum LN VEGF levels and total 24 hour urinary VEGF. Several previous studies have also reported a lack of correlation between circulating and urinary VEGF levels both in healthy subjects [24] and in young patients with renal disease [25]. Although it cannot be concluded that the kidney is the source of increased urine VEGF in the current study, based on lack of correlation between serum or plasma levels and urinary VEGF, previous studies have implicated increased production in the kidney as the source of urine VEGF [26,27]. VEGF is essential for maintenance of glomerular structure [28], endothelial survival and repair in glomerular diseases [29]. The application of proangiogenic factors has been shown to repair the microvasculature and ameliorate chronic kidney disease [30,31]. Thus the fact that higher levels of LN urine VEGF at baseline correlated with a greater decrement in LN albumin excretion might be indicative of a beneficial compensatory repair mechanism in response to the ongoing damage of the kidney. Albuminuria is often an indication of generalized vascular dysfunction. In ADPKD, albuminuria is associated with increased blood pressure and larger renal volume in adults and children [32,33].

VEGF and its receptors play a role in cardiovascular development [34,35]. Cardiomyocytes express VEGF receptors and VEGF has been shown to induce either cardiac hypertrophy or its regression depending on the receptors that it binds to [14]. In the current study, serum VEGF was shown to positively correlate with a larger increase in LVMI indicating a detrimental role of VEGF on cardiac structure in ADPKD. This relationship was present in the absence of cardiac hypertrophy thus might serve as early biomarker for the risk of cardiac complication in ADPKD.

Conclusion

Our present results suggest higher serum VEGF, TSP1 or SPARC levels predict a more rapid decrease in renal function in young patients with ADPKD. While higher serum VEGF predicts an increase in LVMI. In contrast higher urinary VEGF excretion and associated fall in urine albumin may be indicative of ongoing renal repair. Certain limitations with regard to the current study should be noted including the fact that only serum samples were available for assay rather than plasma where serum might have contained growth factors released from platelets. Future analysis of larger cohorts will be necessary to fully evaluate the utility of VEGF and related factors as robust predictors of ADPKD progression.

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Conflict of Interest

None.

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