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The association of angiogenic factors and chronic kidney disease

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Abstract

Background: There are limited data on the associations of circulating angiogenic factors with chronic kidney disease (CKD). We investigate the associations of circulating vascular endothelial growth factor (VEGF)-A, angiopoietin-1, angiopoietin-1/VEGF-A ratio, VEGF receptor 1 (VEGFR-1), VEGFR-2, and pentraxin-3 with CKD.

Methods: We recruited 201 patients with CKD and 201 community controls without CKD from the greater New Orleans area. CKD was defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² or presence of albuminuria. Multivariable quantile and logistic regression models were used to examine the relationship between angiogenesis-related factors and CKD adjusting for confounding factors.

Results: After adjusting for covariables including traditional cardiovascular disease (CVD) risk factors, C-reactive protein, and history of CVD, the medians (interquartile range) were 133.08 (90.39, 204.15) in patients with CKD vs. 114.17 (72.45, 170.32) pg/mL in controls without CKD ($p = 0.002$ for group difference) for VEGF-A; 3951.2 (2471.9, 6656.6) vs. 4270.5 (2763.7, 6537.2) pg/mL ($p = 0.70$) for angiopoietin-1; 25.87 (18.09, 47.90) vs. 36.55 (25.71, 61.10) ($p = 0.0001$) for angiopoietin-1/VEGF-A ratio; 147.81 (122.94, 168.79) vs. 144.16 (123.74, 168.05) ng/mL ($p = 0.25$) for VEGFR-1; 26.20 (22.67, 29.92) vs. 26.28 (23.10, 29.69) ng/mL ($p = 0.31$) for VEGFR-2; and 1.01 (0.79, 1.49) vs. 0.89 (0.58, 1.18) ng/mL ($p = 0.01$) for pentraxin-3, respectively. In addition, an elevated VEGF-A level and decreased angiopoietin-1/VEGF-A ratio were associated with increased odds of CKD.

Conclusions: These data indicate that plasma VEGF-A and pentraxin-3 levels were increased and the angiopoietin-1/VEGF-A ratio was decreased in patients with CKD. Future prospective studies are warranted to examine whether angiogenic factors play a role in progression of CKD.

Keywords: Angiopoietin-1, Vascular endothelial growth factor-a, Pentraxin-3, Chronic kidney disease

Background

Chronic Kidney Disease (CKD) is a highly prevalent disease, affecting over 26.3 million adults in the US alone and 497.5 million in the world [1, 2]. CKD has been associated with increased risks of end-stage renal disease (ESRD), cardiovascular disease (CVD), and premature death [3, 4]. Traditional risk factors only partially explain excess risk of CKD and associated ESRD and CVD in the general

populations [5]. Identification of novel risk factors for CKD will further the understanding of CKD pathogenesis and provide additional targets for therapies [6, 7].

Animal studies have suggested the involvement of an imbalance of angiogenic factors in the pathogenesis of kidney disease [8–14]. In experimental studies, treatment with angiopoietin-1 reduced tubular injury in unilateral ureteral obstruction [15], decreased albuminuria in streptozotocin-induced type-1 diabetes [16], and stabilized peritubular capillaries in folic acid nephropathy, though this was accompanied by profibrotic and inflammatory effects [17]. Deletion of angiopoietin-1 coupled with microvascular stress resulted in organ damage, accelerated angiogenesis and fibrosis [18].

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The findings regarding the association of angiogenic factors and CKD in human are somewhat inconsistent likely due to diversities in sample size, study population, sources of angiogenic factors, and covariables used in the analyses. It was recently reported that vascular endothelial growth factor (VEGF)-A predicted CKD progression in diabetic patients in a small cohort study [19]. However, another study suggested VEGF expression was reduced in the biopsied kidney tissue from patients with diabetic nephropathy [20]. Elevated soluble VEGF receptor-1 (sVEGFR-1) and reduced VEGFR-2 were associated with mortality in dialysis patients [21, 22]. Angiopoietin-1 mediates migration, adhesion, and survival of endothelial cells, and co-expression of angiopoietin-1 and VEGF enhances angiogenesis [23]. Decreased angiopoietin-1 and increased angiopoietin-2 levels have been identified in patients with CKD [24, 25]. Associations of angiopoietin-2 [24–27] and angiopoietin-1 [27] with subclinical CVD have been reported in CKD. Angiopoietin-2 was found to be associated with increased mortality among CKD patients [24]. Pentraxin-3 can bind fibroblast growth factor-2 (FGF2) and act as a FGF2 antagonist to inhibit FGF2-dependent angiogenesis [26].

This study aims to examine the association between multiple circulating angiogenic factors and CKD in a larger pre-dialysis CKD population.

Methods

Study participants

Two hundred one patients with CKD and 201 controls without CKD were recruited between 2007 and 2010 in the greater New Orleans area. The patients with CKD were recruited from nephrology and internal medicine clinics by trained research staff in the study area. These patients were between 21 and 74 years of age. All of the eligible CKD cases identified through the referral clinics were invited to participate. CKD was defined as an eGFR < 60 ml/min/1.73m² or presence of albuminuria (> 30 mg/24-h). Exclusion criteria were a history of chronic dialysis, acute kidney injury, kidney transplant, pregnancy, immunotherapy in the preceding 6 months, chemotherapy in the preceding 2 years, HIV or AIDS, being unable or unwilling to provide informed consent, and participating in a current clinical trial that might have an impact on CKD. Controls were recruited through mass mailing to residents between 21 and 74 years of age residing in the same area, determined by zip code. Control eligibility for participation was assessed by a clinic screening visit.

The Institutional Review Board of Tulane University approved the conduct of this study, and written informed consent was obtained at the screening visit from all participants.

Data collection

Trained staff administered a questionnaire at a clinical visit to obtain demographic information, lifestyle factors

(e.g., cigarette smoking, alcohol consumption, and physical activity), medical history (CVD, diabetes, hypercholesterolemia and hypertension), and the use of medications including aspirin and antihypertensive, hypoglycemic, and lipid-lowering agents.

Three blood pressure (BP) measurements were obtained by trained and certified staff at a clinical visit according to a standard protocol adapted from American Heart Association recommendations [27]. BP was measured using a standard mercury sphygmomanometer, with one of four cuff sizes based on the patient's arm circumference, on the patient in a seated position and after 5 min of rest. Height and weight were measured twice in patients in lightweight indoor clothing without shoes during the clinical visit and were used to calculate body mass index (BMI).

An overnight fasting blood sample was collected to measure glucose, creatinine, cholesterol, triglycerides, and angiogenesis-related biomarkers. Samples were stored at – 80 degrees Celsius (– 80 °C). All samples were measured after being stored for less than 5 years. All of the biomarkers have been previously reported to be stable when stored at – 80 °C [28, 29]. Multiple freeze thaw cycles can increase concentrations of VEGF-A [30]. Biomarkers were measured after the first thaw of the samples to minimize the opportunity for freeze-thaw cycle related changes. Serum creatinine was measured using the Roche enzymatic method (Hoffman-La Roche, Basel, Switzerland). eGFR was estimated based on serum creatinine (SCr), sex, age, and race using the CKD-Epi equation [31]. A 24-h urinary sample was collected to measure creatinine and albumin. Serum cholesterol and triglyceride levels were assayed using an enzymatic procedure on the Hitachi 902 automatic analyzer (Roche Diagnostics, Indianapolis, IN, USA). Serum glucose was measured using a hexokinase enzymatic method (Roche Diagnostics, Indianapolis, IN, USA). Urinary concentrations of albumin and creatinine were measured with a DCA 2000 Analyzer (Bayer AG, Leverkusen, Germany). Plasma VEGF-A, sVEGFR-1, VEGFR-2, and angiopoietin-1 were measured using a sandwich immunoassay on a Meso Scale Discovery Instrument (Meso Scale Diagnostics, LLC., Rockville, MD, USA). Plasma pentraxin-3 was measured by the ELISA assay from R & D Systems (Minneapolis, MN, USA). A stringent quality control process was applied in all laboratory tests. All biomarkers were measured in duplicate, with inter-assay coefficients of variation 23.4% for VEGF-A, 2.4% for sVEGFR-1, 2.6% for VEGFR-2, 10.13% for angiopoietin-1, and 7.1% for pentraxin-3, respectively. All laboratory measures were conducted at the Laboratory for Clinical Biochemistry Research, the University of Vermont.

Statistical analysis

Characteristics of CKD cases and non-CKD controls were compared using Chi-square tests for categorical

variables and t-tests for the continuous variables. Medians and interquartile ranges of the angiogenesis-related biomarkers were calculated for the CKD patients and controls and the differences were compared using the Mann-Whitney test [32]. Quantile regression was used to obtain adjusted-medians and interquartile ranges [33]. The Wald test was used to assess differences in the adjusted medians between CKD patients and controls [33]. The covariates included in the multivariable quantile regression model were age, race, gender, current cigarette smoking, weekly alcohol consumption, physical activity, BMI, LDL-cholesterol, HDL-cholesterol, C-reactive protein, fasting plasma glucose, systolic BP, self-reported history of CVD, and use of aspirin and hypoglycemic, antihypertensive, and lipid-lowering agents.

Multivariable logistic regression models were used to assess adjusted-odds ratios comparing the highest tertile of angiogenesis-related biomarkers to the lower two tertiles between CKD patients and the controls (except for angiotensin-1/VEGF-A ratio, in which the lowest tertile

was compared to the higher two). The same panel of covariates used in the multivariable quantile regression was included in the multivariable logistic regression models. This analysis was also performed stratified by diabetes status.

Associations between the angiogenesis biomarkers and stage of CKD were assessed using polytomous logistic regression and quantile regression. Stages 4 and 5 were combined due to small sample size in each category.

A sensitivity analysis was performed among CKD participants in which the medians of the angiogenesis-related biomarkers were compared between diabetic CKD cases and non-diabetic CKD cases.

Results

Characteristics of the study participants are presented in Table 1. Those with CKD were older, more likely to report a history of CVD, hypertension, diabetes, and dyslipidemia, and to have self-reported use of antihypertensive, hypoglycemic, lipid-lowering agents, or aspirin, and were less likely to drink alcohol, have a high-school education,

Table 1 Characteristics of 201 patients with chronic kidney disease and 201 controls without chronic kidney disease

	CKD Patients (N = 201)	Non-CKD (N = 201)	P-value
Age, years	55.9 ± 9.9	52.5 ± 10.0	< 0.001
Male, %	55.2	45.3	0.06
African-American, %	60.7	51.2	0.06
Current cigarette smoking, %	53.7	48.8	0.32
Alcohol consumption, %	27.9	59.2	< 0.001
Physical activity ≥ twice/week, %	53.0	72.9	< 0.001
High school education, %	58.5	81.6	< 0.001
History of CVD, %	43.7	7.0	< 0.001
History of hypertension, %	88.1	23.9	< 0.001
History of diabetes, %	49.3	5.5	< 0.001
History of hypercholesterolemia, %	65.7	30.9	< 0.001
Use of antihypertensive agents, %	79.6	15.4	< 0.001
Use of hypoglycemic agents, %	34.3	3.0	< 0.001
Use of lipid lowering agents, %	21.9	8.9	< 0.001
Use of aspirin, %	34.8	8.5	< 0.001
BMI, kg/m ²	32.2 ± 7.8	28.9 ± 6.4	< 0.001
Systolic BP, mm Hg	132.2 ± 21.0	122.0 ± 14.7	< 0.001
Diastolic BP, mm Hg	77.2 ± 13.5	77.6 ± 9.4	0.77
LDL-cholesterol, mg/dL	101.8 ± 47.3	118.2 ± 30.2	< 0.001
HDL-cholesterol, mg/dL	50.3 ± 15.6	57.7 ± 18.0	< 0.001
Fasting plasma glucose, mg/dL	119.9 ± 46.8	103.4 ± 35.4	< 0.001
C-reactive protein mg/L	5.3 ± 11.7	4.1 ± 8.4	0.26
eGFR, mL/min/1.73 m ²	43.3 ± 19.3	96.7 ± 16.8	< 0.001
Urinary albumin, mg/24 h	74.5 (12.3, 417.4)	5.9 (4.1, 11.4)	< 0.001

Categorical variables are presented as percentages while continuous variables are presented as mean and standard deviation or median and interquartile range
LDL low-density lipoprotein, HDL high-density lipoprotein, eGFR estimated glomerular filtration rate, BMI body mass index, BP blood pressure, CVD cardiovascular disease

or be physically active. Those with CKD had higher average BMI, systolic BP, and fasting glucose, but lower LDL-cholesterol and HDL-cholesterol.

The age-gender-race adjusted and multivariable-adjusted medians of the angiogenesis-related biomarkers are presented in Table 2. After adjusting for potential confounding factors, the medians of VEGF-A and pentraxin-3 were significantly higher in CKD patients than controls while that of the angiotensin-1/VEGF-A ratio was significantly lower in CKD patients than in controls. The medians of angiotensin-1, VEGFR-1, and VEGFR-2 were not significantly different between CKD patients and controls.

In multivariable logistic regression analysis adjusting for important confounding factors, the odds of CKD were more than doubled for subjects with the highest tertile of VEGF-A compared to those in the lower two tertiles (Table 3). In addition, the odds of CKD were more than three times greater for subjects with the lowest tertile of the angiotensin-1/VEGF-A ratio compared to those in the higher two tertiles. The levels of angiotensin-1, VEGFR-1, VEGFR-2 and pentraxin-3 were not significantly associated with increased odds of CKD in the multivariable analysis.

In multivariable adjusted logistic regression models stratified by diabetes status, strong but non-significant associations were observed for the highest tertile of VEGF-A (OR(95% CI) 6.47(0.89–47.1), $p = 0.07$), lowest tertile of the angiotensin-1/VEGF-A ratio (OR (95% CI) 3.47(0.50–23.9), $p = 0.21$), and the highest tertile of PTX-3 (OR (95% CI) 8.03(0.93–69.6), $p = 0.06$) in diabetics (see Additional file 1: Table S1). Among non-diabetic subjects, a non-significant association was observed for the highest tertile of VEGF-A (OR (95% CI) 1.61 (0.89–2.92), $p = 0.12$), while significant associations were observed for the lowest tertile of the angiotensin-1/VEGF-A ratio (OR (95% CI) 3.02 (1.64–5.57), $p = 0.0004$) and the highest tertile of PTX-3 (OR (95% CI) 1.98 (1.06–3.69), $p = 0.03$). No significant associations were observed for angiotensin-1, VEGFR-1

or VEGFR-2 in multivariable adjusted models in diabetic or non-diabetic subjects.

In multivariable adjusted quantile regression models stratified by stage of CKD, median VEGF-A significantly increased with increasing severity of CKD (Table 4). Median angiotensin-1/VEGF-A ratio significantly decreased with increasing CKD severity. PTX-3 increased with increasing CKD severity, though this did not achieve statistical significance. VEGFR-1 level increased significantly with increasing severity of CKD. Medians for angiotensin-1 and VEGFR-2 did not differ by stage of CKD.

In multivariable adjusted polytomous logistic regression models, the association between high VEGF-A and CKD was observed for both stage 3 and 4/5 (OR (95% CI) 2.75(1.34–5.64) and 1.62(0.64–4.11), respectively, $p = 0.02$). The association between low angiotensin-1/VEGF-A ratio and CKD was observed for both stage 3 and stage 4/5 (OR (95% CI) 3.07 (1.49–6.32) and 6.17 (2.48–15.3) respectively, $p = 0.0003$). A non-significant association between elevated PTX-3 and CKD was observed for both stage 3 and stage 4/5 CKD (OR (95% CI) 1.58(0.76–3.31) and 2.15(0.86–5.37), respectively, $p = 0.2$). No association was observed between high VEGFR-1 and stage 3 CKD, but a significant association was observed for stage 4/5 (OR (95% CI) 0.99(0.46–2.10) and 3.01(1.19–7.58), respectively, $p = 0.02$). No association was observed for angiotensin-1 or VEGFR-2.

In the sensitivity analysis, medians of the biomarkers were compared for diabetic and non-diabetic cases. There was no significant difference in the medians of the biomarkers between the diabetic and non-diabetic CKD patients for angiotensin-1, VEGF-A, angiotensin-1/VEGF-A ratio, VEGFR-1, VEGFR-2, or pentraxin-3.

Discussion

The present study indicated that higher VEGF-A and pentraxin-3 levels and a lower angiotensin-1/VEGF-A ratio may be associated with increased risk of CKD. These associations remained after adjustment for established CKD risk factors as well as CVD and the use

Table 2 Angiogenesis-related factors according to chronic kidney disease status

	Age-gender-race-adjusted median (IQR)			Multivariable-adjusted median (IQR) ^a		
	CKD patients (n = 201)	Non-CKD controls (n = 201)	P	CKD patients (n = 201)	Non-CKD controls (n = 201)	P
VEGF-A, pg/mL	132.6 (90.4, 199.0)	112.5 (71.9, 166.8)	0.13	133.08 (90.39, 204.15)	114.17 (72.45, 170.32)	0.002
Angiotensin-1, pg/mL	3957.1 (2471.9, 6602.1)	4269.1 (2668.5, 6501.9)	0.34	3951.2 (2471.9, 6656.6)	4270.5 (2763.7, 6537.2)	0.70
Angiotensin-1/VEGF-A	25.80 (18.09, 47.90)	36.69 (25.71, 61.10)	< 0.001	25.87 (18.09, 47.90)	36.55 (25.71, 61.10)	< 0.001
VEGFR-1, ng/mL	148.0 (122.9, 167.9)	144.2 (123.7, 168.0)	0.92	147.81 (122.94, 168.79)	144.16 (123.74, 168.05)	0.25
VEGFR-2, ng/mL	26.1 (22.7, 29.9)	26.4 (23.1, 29.7)	0.79	26.20 (22.67, 29.92)	26.28 (23.10, 29.69)	0.31
Pentraxin-3, ng/mL	1.02 (0.79, 1.48)	0.86 (0.58, 1.17)	0.01	1.01 (0.79, 1.49)	0.89 (0.58, 1.18)	0.01

VEGF-A vascular endothelial growth factor A; VEGFR-1 = vascular endothelial growth factor receptor 1; VEGFR-2 = vascular endothelial growth factor receptor 2; CKD = chronic kidney disease; IQR = interquartile range

^aMultivariable adjusted model adjusted for age, race, gender, current cigarette smoking, weekly alcohol consumption, physical activity \geq twice/week, BMI, LDL-cholesterol, HDL-cholesterol, C-reactive protein, fasting plasma glucose, systolic BP, use of aspirin or lipid-lowering, antihypertensive, or antidiabetic medications, and history of CVD

Table 3 Multivariable-adjusted odds ratios of chronic kidney disease by Dichotomized^a Angiogenesis-related Factors

	Age, gender, and race-adjusted			Multivariable-adjusted ^b		
	Odds ratios	95% CI	<i>p</i> -value	Odds ratios	95% CI	<i>p</i> -value
VEGF-A ≥ 160.7 pg/mL	1.84	1.17–2.90	0.01	2.40	1.20–4.81	0.01
Angiotensin-1 ≥ 5659.5 pg/mL	0.87	0.55–1.36	0.54	1.15	0.57–2.32	0.70
Angiotensin-1/VEGF-A ≤ 24.2	3.19	2.00–5.10	< 0.001	3.59	1.80–7.18	0.0003
VEGFR-1 ≥ 159.7 ng/mL	1.10	0.70–1.75	0.68	1.36	0.67–2.75	0.40
VEGFR-2 ≥ 28.3 ng/ml	1.28	0.81–2.01	0.29	1.11	0.55–2.22	0.78
Pentraxin-3 ≥ 1.13 ng/mL	1.86	1.18–2.94	0.008	1.74	0.86–3.52	0.13

^aDichotomized as upper tertile compared to lower two tertiles for all biomarkers except the ratio of angiotensin-1/VEGF-A, which was dichotomized as lowest tertile compared to upper two tertiles

^bAdjusted for age, race, gender, current cigarette smoking, weekly alcohol consumption, physical activity ≥twice/week, BMI, LDL-cholesterol, HDL-cholesterol, C-reactive protein, fasting plasma glucose, systolic BP, use of aspirin or lipid-lowering, antihypertensive, or antidiabetic medications, and history of CVD

of antihypertensive, antidiabetic, lipid-lowering medications, and aspirin. These findings suggest that abnormal angiogenesis is present in patients with CKD.

Our study reports that plasma VEGF-A is significantly higher in patients with pre-dialysis CKD compared to controls. Animal and laboratory studies have suggested that increased VEGF-A expression causes glomerular hypertrophy, proliferation of podocytes, mesangial cell proliferation, extracellular matrix expansion, interstitial fibrosis, and proteinuria [34, 35]. The therapeutic effects of anti-VEGF-A and anti-angiogenic factors in experimental diabetic nephropathy have been reported, including amelioration of increases in urinary albumin excretion, glomerular volume, glomerular basement membrane thickening, in addition to decreased slit pore density and nephrin quantity [36–38]. Urinary VEGF was reported to be elevated in patients with diabetic nephropathy and positively associated with proteinuria [39]. Furthermore, plasma VEGF-A levels have previously been found to be associated with progression to ESRD in 67 patients with diabetic CKD [19]. However, Lindenmeyer et al. reported a decrease in mRNA expression of VEGF-A in the renal interstitium of patients with diabetic nephropathy in a small study [20]. A study of murine folic acid induced nephropathy found depleted VEGF-A in kidney tissue, but increased circulating

VEGF-A, possibly from damage to the systemic vasculature induced by folic acid [17]. Our study findings support the hypothesis that increased circulating VEGF-A may be associated with increased risk of CKD. Further studies are warranted to examine the causal relationship of VEGF-A and the progression of CKD. Additionally, our study suggests that the treatment targeting VEGF in CKD needs further careful evaluation due to inconsistency between increased circulating VEGF-A and decreased renal expression of VEGF-A in findings from different studies.

Our study identified lower angiotensin-1 in CKD patients than non-CKD controls, and lower angiotensin-1 with increased CKD severity, though these differences did not achieve statistical significance. Decreased angiotensin-1 has been reported in pre-dialysis CKD in children [25], but was not associated with eGFR [40] or mortality among patients with CKD [24]. Animal studies indicate that treatment with angiotensin-1 might reduce kidney damage in unilateral ureteral obstruction, streptozotocin-induced type-1 diabetes, and folic acid induced nephropathy [15–17]. Deletion of angiotensin-1 from mice embryos coupled with injury or microvascular stress caused organ damage, accelerated angiogenesis and fibrosis, suggesting angiotensin-1 may balance the angio-fibrogenic response associated with elevated VEGF-A and angiotensin-2 levels from tissue injury and microvascular disease, like

Table 4 Angiogenesis-related factors according to chronic kidney disease stage

	Multivariable-adjusted median (IQR) ^a			<i>p</i> -value
	Non-CKD controls (N = 201)	CKD Stage 3 (N = 142)	CKD Stage 4/5 (N = 59)	
VEGF-A, pg/mL	114.17 (72.45, 170.32)	128.71 (89.19, 214.49)	134.27 (95.73, 178.73)	0.001
Angiotensin-1, pg/mL	4270.5 (2763.7, 6537.2)	4026.3 (2511.6, 6881.9)	3753.5 (2423.0, 6181.4)	0.44
Angiotensin-1/VEGF-A	36.55 (25.71, 61.10)	26.36 (18.82, 50.19)	23.47 (16.83, 42.51)	0.001
VEGFR-1, ng/mL	144.16 (123.74, 168.05)	135.96 (118.60, 160.40)	163.96 (135.19, 184.76)	0.04
VEGFR-2, ng/mL	26.28 (23.10, 29.69)	25.78 (22.23, 29.68)	27.85 (23.12, 31.10)	0.19
Pentraxin-3, ng/mL	0.89 (0.58, 1.18)	1.01 (0.79, 1.38)	1.04 (0.78, 1.58)	0.06

^aAdjusted for age, race, gender, current cigarette smoking, weekly alcohol consumption, physical activity ≥twice/week, BMI, LDL-cholesterol, HDL-cholesterol, C-reactive protein, fasting plasma glucose, systolic BP, use of aspirin or lipid-lowering, antihypertensive, or antidiabetic medications, and history of CVD

that observed in diabetes [18]. More studies are warranted to confirm the relationship of angiotensin-1 and CKD in humans.

A low ratio of angiotensin-1 to VEGF-A was significantly associated with odds of CKD in our study. Similar associations were observed in both diabetic and non-diabetic subjects, but the associations achieved significance only in non-diabetic subjects likely due to the small sample size of the diabetic group. Lower angiotensin-1, relative to VEGF-A concentrations, may be associated with impaired angiogenesis and enhanced endothelial leakage induced by VEGF-A as co-expression of angiotensin-1 and VEGF-A enhances angiogenesis [19] and angiotensin-1 can potentially block VEGF-induced endothelial permeability in vitro [41]. Podocyte specific depletion of angiotensin-1 in a model of type 1 diabetes decreased glomerular endothelial cell proliferation, hyperfiltration and albuminuria by 70% [16]. Angiotensin-1 deficiency and VEGF-A excess is thought to destabilize endothelia in type 1 diabetic mice, and the improvements observed in mice treated with angiotensin-1 may be attributable to vascular stabilization from attenuation of VEGF-A signaling by increased angiotensin-1 [16, 18]. Our study sample was found to have a similar growth factor milieu, with angiotensin-1 deficiency relative to excess VEGF-A among CKD patients. A recent study suggested that low angiotensin-1 level was positively associated with abnormal cardiac structure in stages 3–5 CKD patients [42]. Further studies are warranted to investigate whether imbalanced angiotensin-1 and VEGF-A may be associated with an increased risk of ESRD and CVD in CKD patients.

The VEGFR-1 and VEGFR-2 levels were not significantly different between CKD patients and controls. VEGFR-1 was significantly elevated in subjects with stage 4 and 5 CKD compared to the controls, suggesting that high VEGFR-1 may be associated with more severe CKD, which may conform with the observation that elevated VEGFR-1 was associated with inflammation and mortality in dialysis patients in previous studies [21, 22]. Unlike our study, a previous study reported VEGFR-2 to be lower in dialysis patients [22]. The underlying explanation for this inconsistency between our study finding and the other's may be due to differences in the study population and the severity of CKD.

Pentraxin-3 levels were significantly higher in patients with CKD compared to the controls after adjusting for multiple confounding factors including C-reactive protein in our study, even though pentraxin-3 did not increase substantially with increased severity of CKD and the odds of CKD associated with pentraxin-3 did not achieve statistical significance in multivariable logistic analysis. When logistic regression models were run separately in diabetic and non-diabetic subjects, a statistically significant doubling of odds of CKD among those with high pentraxin-3 was observed

in non-diabetic subjects, while a much larger but not statistically significant increase in odds of CKD was observed among diabetic subjects. The inconsistency of these findings is likely due to limited statistical power in the subgroup analyses. Pentraxin-3 has been associated with endothelial dysfunction, decreased eGFR, and proteinuria in previous studies [43, 44]. It is also associated with inflammation in cardiovascular disease [45, 46]. However, the association observed between PTX-3 and CKD is independent of inflammatory and endothelial dysfunction biomarker C-reactive protein in our study, suggesting that PTX-3 might play a role in pathogenesis of CKD via an additional pathway such as abnormal angiogenesis. Future study is needed in this important area.

There are several noteworthy strengths of this study. Our study is among the largest of early studies that have found plasma VEGF-A levels were significantly increased in patients with CKD and that there might be a significant imbalance of VEGF-A and angiotensin-1 in CKD patients. These associations were independent of multiple covariables for CKD that were carefully measured in our study. Furthermore, our study included a racially diverse group of patients with variable degrees of renal function. This study also had several limitations. First, this is a cross-sectional analysis, which prevents the determination of direction of the relationship between these angiogenic factors and CKD. Second, our study has a relatively small sample size. There is limited statistical power to do subgroup analyses by severity of CKD and diabetes status. Third, the majority of our CKD cases have stage 3 CKD, with only a minority in stage 4 or 5. An underrepresentation of more severe CKD may have limited our ability to identify associations between biomarkers and severe CKD. A larger prospective cohort study might provide more definitive evidence for the association of plasma angiogenic factors with CKD.

Conclusions

In conclusion, this study shows that higher circulating VEGF-A and pentraxin-3 levels as well as a lower angiotensin-1/VEGF-A ratio may be associated with an increased risk of CKD. Future prospective studies are warranted to examine whether these angiogenic factors play a role in progression of CKD and these abnormalities of angiogenic factors may serve as therapeutic targets for treatment of CKD.

Additional file

Additional file 1: Table S1. Age, Race and Gender Adjusted and Multivariable-adjusted Odds Ratios of Chronic Kidney Disease in Patients with and without Diabetes by Dichotomized* Angiogenesis-related Factors. The data presented in the table describe the associations between the angiogenesis related factors and CKD in diabetics and non-diabetics. (DOCX 16 kb)

Abbreviations

BMI: Body mass index; BP: Blood pressure; CKD: Chronic kidney disease; CVD: Cardiovascular disease; eGFR: estimated glomerular filtration rate; ESRD: End-stage renal disease; FGF: Fibroblast growth factor; SCR: Serum creatinine; sVEGFR: soluble VEGF receptor; VEGF: Vascular endothelial growth factor; VEGFR: Vascular endothelial growth factor receptor

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to restrictions put in place by the Institutional Review Board for Tulane University, but are available from the corresponding author on reasonable request.

Authors' contributions

CEA, JH and JC participated in interpretation of the data and drafting of the manuscript. GB, DRK, SGK, RS, SG, MK, KK, ES helped in patient recruitment, data collection, and critical review. CEA and CSC performed the data management and statistical analysis. LLH, JH and JC conceived of the study, participated in the design of the study, supervision of the study, and interpretation of the data. JC obtained research funding for the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Review Board of Tulane University approved the conduct of this study, and written informed consent was obtained at the screening visit from all participants.

Competing interests

The authors declare that they have no competing interests.

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