Correlates of Resistin in Children with Chronic Kidney Disease: The Chronic Kidney Disease in Children Cohort

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Objective To test the hypothesis that resistin is associated with insulin resistance and inflammation in pediatric patients with chronic kidney disease (CKD).

Study design This study is a cross-sectional analysis of 319 children in the Chronic Kidney Disease in Children cohort, a large cohort of children with stage II-IV CKD. Univariate and multivariate regression modeling was used to evaluate the association of serum resistin level with glomerular filtration rate (GFR), demographic data, and cardiovascular risk factors, including inflammatory cytokines, insulin resistance, and serum lipids.

Results In univariate analyses, serum resistin level was negatively correlated with GFR ($P < .01$). Increased serum resistin was associated with elevated inflammatory cytokines, including interleukin (IL)-6 ($P < .01$), IL-10 ($P < .01$), and tumor necrosis factor-α ($P < .01$). Resistin level was not associated with insulin resistance, although it was positively correlated with serum triglycerides ($P < .01$) and negatively correlated with high-density lipoprotein cholesterol ($P < .01$). In multivariate analysis, GFR ($\beta = -0.01$; $P < .001$), IL-6 ($\beta = 0.18$; $P < .001$), IL-10 ($\beta = 0.09$; $P = .01$), and pubertal status ($\beta = 0.18$; $P < .01$) were significantly associated with serum resistin level.

Conclusion These results indicate that serum resistin level increases with GFR decline and is involved in the inflammatory milieu present in CKD. *(J Pediatr 2012;161:276-80)*.

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in children and young adults with end-stage renal disease.[1] Given that CVD in children is often subclinical, biomarkers predictive of cardiovascular morbidity are needed to improve long-term outcomes in pediatric patients with chronic kidney disease (CKD). Resistin is a 12.5-kDa protein belonging to a family of cysteine-rich proteins known as resistin-like molecules. Since its discovery in 2001, resistin has generated much interest because of its association with known risk factors for CVD, including insulin resistance[2-4] and inflammation.[5-9] Therefore, it has been suggested that elevated serum resistin level may represent a novel risk factor for CVD.[10]

Serum resistin levels are elevated in patients with CKD.[5,6,11,12] The few studies performed in adults with CKD have failed to identify a relationship between serum resistin level and insulin resistance[6,7]; however, resistin has been found to be associated with tumor necrosis factor (TNF)-α[6,7] and interleukin (IL)-6,[5,6,8,9] suggesting that the proinflammatory effects of resistin may be more important in increasing cardiovascular risk.

In the present study, we hypothesized that resistin is associated with insulin resistance and inflammation in pediatric patients with renal insufficiency. We tested this hypothesis in a cross-sectional study of patients enrolled in the Chronic Kidney Disease in Children (CKiD) study, a prospective observational study of children with mild to moderate CKD. We analyzed the association between resistin level and markers of insulin resistance and inflammation, as well as other potential CVD risk factors in children with CKD.

Methods

The CKiD study is a longitudinal, observational study of a cohort of 586 children with CKD conducted at 46 pediatric nephrology centers in North America. Institutional Review Board approval was obtained at each participating center. Specific details on the design and methods of this study have been published previously.[13] The goals of the CKiD study include to identify risk factors for the progression of CKD as well as to describe the prevalence of novel and traditional risk factors for CVD in this patient population. Inclusion criteria were age 1-16 years and a

BMI	Body mass index
CKiD	Chronic Kidney Disease in Children
CKD	Chronic kidney disease
CVD	Cardiovascular disease
GFR	Glomerular filtration rate
HOMA-IR	Homeostasis model assessment of insulin resistance
IL	Interleukin
TNF	Tumor necrosis factor

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glomerular filtration rate (GFR) of 30-90 mL/min/1.73 m^2 as estimated by the Schwartz equation. Among study participants, 319 patients had serum available for analysis of resistin levels. The present study is a cross-sectional analysis of baseline serum resistin levels obtained in this cohort at year 2 of the CKiD study.

Serum resistin analysis was carried out at the Metabolic Phenotyping Core at the University of Texas Southwestern Medical Center, Dallas using serum samples from the CKiD study. Original samples were collected locally, shipped, and stored at −80°C at the National Institutes of Health's Biologic Repository. The Human Resistin ELISA Kit (Millipore, Billerica, Massachusetts) was used to determine total serum resistin.

In addition to resistin, fasting insulin, TNF-α, IL-6, and IL-10 levels from the year 2 visit were determined utilizing the same laboratory. A bead-based multiplex assay (Luminex 100; Bio-Rad, Hercules, California) was used to determine cytokine concentrations. This assay has been validated and shown to have excellent linearity, precision, and sensitivity.[14-16] The homeostasis model assessment of insulin resistance (HOMA-IR) was used as a measure of insulin resistance. HOMA-IR score was calculated by dividing the product of serum insulin (mU/mL) and glucose (mmol/mL) by a factor of 22.5.

Other laboratory data obtained for analysis included fasting lipid profiles (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides) and urine protein-to-creatinine ratio. GFR was calculated using plasma iohexol disappearance curves (iGFR), as described previously.[13] Demographic and clinical data, including age, sex, body mass index (BMI), pubertal status, and casual blood pressure, were collected at the same time that serum resistin levels and other laboratory measurements were obtained.

For statistical analysis, continuous variables are reported as median and IQR, because most variables were not normally distributed. Univariate analyses were performed to explore the relationship between the dependent variable (serum resistin level) with clinical and demographic data. Comparisons between categorical variables were performed using the Wilcoxon rank-sum test or Kruskal-Wallis test. For all continuous variables, Pearson correlation coefficients were calculated along with their associated *P* values to identify variables with a statistically significant association with serum resistin level. Laboratory values, including serum resistin, inflammatory markers, indices of insulin resistance, and lipid profiles, were log-transformed to achieve normality and fulfill assumptions of linear regression modeling. Multivariate regression analysis was performed using all variables with a *P* value of <.10 in univariate analyses. Variables with a *P* value of <.05, selected using backward elimination, were considered statistically significant and included in a final regression model. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

Results

Characteristics of the study cohort are summarized in the Table. The cohort was predominately Caucasian and male; approximately one-half (48%) were prepubertal. Median iGFR was 45 mL/min/1.73m^2. The majority of patients had a nonglomerular cause of CKD. The most common nonglomerular causes of CKD were obstructive uropathy, renal dysplasia, and reflux nephropathy (21%, 17%, and 16% of the entire cohort, respectively). Among glomerular etiologies, focal and segmental glomerulosclerosis and hemolytic uremic syndrome were most prevalent, representing 7% and 4% of the cohort, respectively. Obesity was seen in 15% of the cohort; hypertension, in 15% as well.

Table. Baseline characteristics of the CKiD cohort

Characteristic	CKiD cohort (n = 319)
Age, years	12.4 (8.9, 15.6)
Sex, %	
Male	57
Female	43
Ethnicity, %	
Caucasian	72
African-American	13
Other	15
Cause of CKD, %	
Glomerular	21
Nonglomerular	79
Pubertal status, %	
Prepubertal	48
Pubertal	52
Duration of CKD, years	7.6 (4.3-1.7)
iGFR, mL/min/1.73 m^2	45 (34-58)
Height percentile	25 (8-51)
Weight percentile	45 (21-77)
BMI percentile	60 (33-88)
Systolic blood pressure percentile	59 (29-82)
Diastolic blood pressure percentile	61 (39-85)
HDL, mg/dL	48 (41-56)
LDL, mg/dL	104 (84-121)
Triglycerides, mg/dL	107 (74-143)
IL-6, pg/mL	1.9 (1.1-3.6)
IL-10, pg/mL	2.3 (1.6-4.3)
TNF-α, pg/mL	5.0 (3.6-8.2)
Resistin, ng/mL	18.6 (13.7-27.1)
Glucose, mg/dL	89 (83-95)
Insulin, mU/L	7.6 (4.2-12.2)
HOMA-IR	1.7 (0.9-2.8)
Protein-to-creatinine ratio	0.44 (0.18-1.1)

HDL, high-density lipoprotein; *LDL*, low-density lipoprotein.
Continuous variables are presented as median (IQR); categorical variables, as percentage. Prepubertal is defined as Tanner stage 1; pubertal, as Tanner stage >1.

Serum Resistin Level and Patient Demographics

Serum resistin level did not differ significantly by sex or race. The median serum resistin level was higher in children with a glomerular etiology compared with those with a nonglomerular etiology (21.3 ng/mL vs 17.9 ng/mL; $P = .03$). Pubertal children had higher serum resistin levels than prepubertal children (median 20.3 ng/mL vs 16.7 ng/mL; $P = .01$). Serum resistin level increased with age ($r = 0.15$; $P < .01$). There was no relationship between BMI *z* score based on age and sex and serum resistin level.

Serum Resistin Level, Markers of Inflammation, and Renal Function

In our cohort, serum resistin level was negatively correlated with iGFR (Figure 1) and positively correlated