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ORIGINAL ARTICLE

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Baseline tubular biomarkers in young adults with congenital heart disease as compared to healthy young adults: Detecting subclinical kidney injury

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Abstract

Background: There are significant implications for kidney disease in young adults with congenital heart disease. Prior investigations have not focused on the use of urinary tubular biomarkers for the early identification of kidney disease in this growing patient group.

Objective: Determine if young adults with congenital heart disease have differences in the baseline concentration of urinary tubular biomarkers when compared to healthy young adults.

Design/Methods: In a pilot case control study, 30 patients from 18 to 35 years of age with congenital heart disease and a normal serum creatinine were recruited during a routine follow-up visit. In the same age group, 30 control subjects without history of heart or kidney disease were recruited. Urine samples were obtained to measure beta 2-microglobin, alpha 1-microglobin, N-acetyl-B-D-glucosaminidase, liver fatty acid binding protein, kidney injury molecule-1, insulin-like growth factor binding protein 7, and tissue inhibitor of metalloproteinases-2. Comparisons were done using Wilcoxon rank-sum or Fisher's exact test.

Results: No study participants had proteinuria on urine dipstick. Median concentrations of kidney injury molecule-1 were higher ($P = .01$) and concentrations of insulin-like growth factor binding protein 7 ($P = .001$) and tissue inhibitor of metalloproteinases-2 ($P = .009$) were lower in the subjects with congenital heart disease when compared to the control subjects. There were no significant differences between the groups with respect to the other biomarkers.

Conclusion: Our data suggest that young adults with congenital heart disease may have subclinical kidney dysfunction. Lower levels of insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2 may indicate an impaired ability to respond to injury, while higher levels of kidney injury molecule-1 may reflect early tubular injury.

KEYWORDS

congenital heart disease, kidney injury, tubular injury markers, urinary biomarkers

1 | INTRODUCTION

Adults with congenital heart disease are at an increased risk of chronic kidney disease and its associated morbidity and mortality when compared to the general population.[1] Commonly, patients are diagnosed with kidney disease during the sporadic monitoring of serum creatinine or a urinalysis, which are late indicators of renal dysfunction. There are no clear guidelines on the renal evaluation during routine clinic visits or preoperatively for these patients. Given that, tubulointerstial damage has been shown to play a role in the early progression to chronic kidney disease,[2-4] urinary tubular injury biomarkers have been explored previously in children with congenital heart disease. Differences have been demonstrated in the baseline concentration of tubular biomarkers in children with congenital heart disease when compared to healthy age matched controls, including beta 2-microglobin,[5] alpha 1-microglobin,[6] N-acetyl-B-D-glucosaminidase,[7] liver fatty acid binding protein,[8] and kidney injury molecule-1.[8]

Urinary insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2 are markers of cell cycle arrest that are thought to act as a signal for early cellular stress.[2,9] Although these newer urinary tubular biomarkers have been validated for acute kidney injury risk assessment in children and adults, there is little published data on baseline levels. Lower baseline levels of the product of tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 in older adults as well as in patients with chronic kidney disease have been reported.[10,11] These results led us to hypothesize that lower values of tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 may indicate a poor ability to respond to cellular stress. No prior studies have explored the use of tubular biomarkers specifically in young adults with congenital heart disease in the evaluation of their renal health. Importantly, age can have an impact on urinary biomarker values.[12] We sought to determine if young adults with congenital heart disease have differences in the baseline concentration of tubular biomarkers when compared to healthy young adults.

2 | METHODS

We performed a pilot case control study of the first 30 eligible patients from 18 to 35 years of age with congenital heart disease presenting for the routine follow-up at the Children's Hospital of Pittsburgh Adult Congenital Heart Disease Center between February 2017 and September 2017. The study was approved by the institutional review board of the University of Pittsburgh and informed consent was obtained on all participants. Eligible patients had a creatinine in the last year of ≤ 1.1 mg/dl for females and ≤ 1.3 mg/dl for males. Patients were excluded if they had a known history of urologic disease, dialysis, or kidney transplant. Data regarding the type of congenital heart disease, blood pressure at the time of the study visit, current medications, and most recent transthoracic echocardiogram result were obtained from the medical record. Subjects between the ages of 18 and 35 were recruited from the University of Pittsburgh Campus. Control subjects were excluded if they had a known history of cardiac disease, kidney disease, transplantation, urologic disease, or dialysis.

No subjects participated in a contrast study or an exercise test within two days of urine sampling. All study participants provided at least 30 mL of urine on the day of the clinic or study visit. Using a dipstick test, protein was quantified on all urine samples. Urine albumin was measured using an immunoturbidimetric assay. Samples were centrifuged at 2000*g* for 10 minutes and the supernatants were stored in a −20°C freezer until analysis. Urinary tissue inhibitor of metalloproteinases-2, insulin-like growth factor binding protein 7 and the product of tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 ([tissue inhibitor of metalloproteinases-2]•[insulin-like growth factor binding protein 7]) was measured with the NephroCheck™ Test (Astute Medical, San Diego, CA, USA). Urine values of liver fatty acid binding protein (Millipore, Temecula, CA, USA), kidney injury moleclue-1 (Millipore, Temecula, CA, USA), alpha 1-microglobin (Millipore, Temecula, CA, USA), N-acetyl-B-D-glucosaminidase (MyBiosource, San Diego, CA, USA), and beta 2-microglobin (Quest Diagnostics, Chantily, VA, USA) were measured according to the manufacturer's protocol. Samples for albumin, liver fatty acid binding protein, kidney injury molecule-1, alpha 1-microglobin, N-acetyl-B-D-glucosaminidase, and beta 2-microglobin were corrected for urinary creatinine. Automated blood pressure readings were used to calculate systolic and diastolic blood pressure stages according to the 2017 American College of Cardiology/American Heart Association guidelines.[13]

Data are presented as median, percentage, or interquartile range. Differences between cases and controls were determined using the nonparametric Wilcoxon rank-sum test or Fisher's exact test. A two-sided *P* value of .05 was used to indicate statistical significance. The analysis was done using SAS version 9.3 (SAS Institute, Cary, NC, USA).

3 | RESULTS

Thirty adults with a diagnosis of congenital heart disease and 30 adults without a diagnosis of congenital heart disease participated in the study. All study participants had no clinically significant proteinuria on urine dipstick. Table 1 depicts the characteristics of the participants at the time of the study visit. The urine albumin to creatinine ratio was similar when comparing those with congenital heart disease to those without congenital heart disease ($P = .15$). When we defined albuminuria as a urine albumin to creatinine ratio of 30 mg/g or more, two healthy young adults and four subjects with congenital heart disease met the criteria for albuminuria ($P = .67$). The median age was similar when comparing the subjects with congenital heart disease (28 years) to subjects without congenital heart disease (27 years), $P = .17$. Twenty males with congenital heart disease and 17 males without congenital heart disease participated in the study ($P = .59$). All patients with congenital heart disease had

TABLE 1 Characteristics of cases and controls

Characteristic	Cases	Controls	P Value
Age in years, median (IQR)	28 (26-31)	27 (22-30)	.17
Male Gender, n (%)	20 (66.7)	17 (56.7)	.59
Albumin to Creatinine Ratio (mg/g)	9.51 (4.79-18.94)	5.67 (2.94-18.31)	.15
Normal Blood Pressure,[a] n (%)	12 (40.0)		
Elevated Blood Pressure,[a] n (%)	18 (60.0)		
BMI (kg/m^2), median (IQR)	20.2 (18.3-21.4)		
Serum Creatinine (mg/dL), median (IQR)	0.9 (0.8-1.0)		
Estimated Glomerular Filtration Rate (mL/min/1.73 m^2),[b] median (IQR)	103.0 (87.3-118.3)		
Ejection Fraction (%), median (IQR)	56.7 (49.0-62.0)		

Abbreviation: IQR, Interquartile Range.

[a]Based on the 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines.[13]

[b]Based on the Chronic Kidney Disease-Epidemiology Collaboration equation.[14]

TABLE 2 Type of congenital heart disease (n = 30)

Primary diagnosis	n (%)
D-transposition of the great arteries	6 (20.0)
Bicuspid aortic valve	5 (16.7)
Tetralolgy of Fallot	5 (16.7)
Pulmonary valve stenosis	4 (13.3)
Coarctation of the aorta	3 (10.0)
Complex congenital heart disease status post Fontan palliation	3 (10.0)
Complete atrioventricular septal defect	2 (6.7)
Aortic stenosis	1 (3.3)
Ventricular septal defect	1 (3.3)

normal kidney function by conventional markers, including a normal serum creatinine and estimated glomerular filtration rate.[14] No subjects had stage 1 or 2 hypertension. Table 2 shows the represented congenital heart disease diagnoses. The most common diagnosis was D-transposition of the great arteries with all subjects with this diagnosis having undergone an arterial switch operation. All patients had an oxygen saturation >90% at the time of their visit. Patients with congenital heart disease reported taking a variety of medications (Table 3). Table 4 shows the differences in urinary tubular biomarkers when comparing groups. Urinary levels of kidney injury molecule-1/creatinine were significantly higher in subjects with congenital heart disease when compared to those without congenital heart disease (576.50 pg/mg vs 332.77 pg/mg, P = .01). Urinary concentrations of tissue inhibitor of metalloproteinases-2 (2.50 ng/mL vs 3.70 ng/mL, P = .009) and insulin-like growth factor binding protein 7 (33.50 ng/mL vs 69.75 ng/mL, P = .001) were significantly lower in patients with a congenital heart disease when compared to subjects without a diagnosis.

TABLE 3 Medications taken by patients with congenital heart disease

Medication	n (%)
Metoprolol	9 (30.0)
Aspirin	5 (16.7)
Warfarin	5 (16.7)
Furosemide	4 (13.3)
Lisinopril	4 (13.3)
Losartan	2 (6.7)
Synthroid	2 (6.7)
Carvedilol	1 (3.3)
Enalapril	1 (3.3)
Metformin	1 (3.3)
Methylphenidate	1 (3.3)
Seroquel	1 (3.3)
Sotalol	1 (3.3)
Cetirizine	1 (3.3)

4 | DISCUSSION

In this pilot study, despite a limited sample size, we found a difference in the concentrations of urinary tubular injury markers kidney injury molecule-1, tissue inhibitor of metalloproteinases-2, and insulin-like growth factor binding protein 7 when comparing young adults with congenital heart disease to healthy young adults of similar age and gender. Importantly, all participants did not have clinically significant proteinuria, there was no significant difference in albuminuria when comparing the two groups, and routine indicators of kidney dysfunction were normal in all patients with congenital heart disease. Initial investigations have focused on kidney injury

TABLE 4 Tubular biomarkers when comparing subject groups (n = 60)

Tubular biomarker	Healthy young adults[a] (n = 30)	Young adults with CHD[a] (n = 30)	*P* value
Alpha 1-microglobin/Cr (mg/g)	11.49 (5.64-23.20)	13.53 (6.93-21.93)	.80
Beta 2- microglobin/Cr (ug/g)	67.24 (44.33-148.03)	74.77 (56.02-127.83)	.69
KIM-1/Cr (pg/mg)	332.77 (206.85-608.00)	576.50 (351.71-996.52)	.01
L-FABP/Cr (ng/mg)	2.53 (1.44-4.81)	2.49 (1.70-5.11)	.720
N-acetyl-B-D-glucosaminidase/Cr (IU/g)	9.54 (6.88-20.33)	13.93 (8.70-23.03)	.16
TIMP-2 (ng/mL)	3.70 (2.85-5.23)	2.50 (2.00-3.94)	.009
IGFBP7 (ng/mL)	69.75 (43.58-93.55)	33.50 (23.88-55.38)	.001
[(TIMP2 • IGFBP7)/1000]	0.28 (0.14-0.44)	0.10 (0.05-0.19)	.0004

Abbreviations: CHD, Congenital Heart Disease; Cr, Creatinine; IGFBP, Insulin-Like Growth Factor Binding Protein; KIM, Kidney Injury Molecule; L-FABP, Liver Fatty Acid-Binding Protein; TIMP, Tissue Inhibitor of Metalloproteinases.

[a]Median (Interquartile Range).

molecule-1, tissue inhibitor of metalloproteinases-2, and insulin-like growth factor binding protein 7 as early indicators of acute kidney injury, however, more recent evidence suggests that they may be used to detect chronic tubular injury as well.

Significant associations between urinary kidney injury molecule-1 and renal fibrosis in human biopsy specimens have been shown.[15] Kaddourah et al reported that children with dilated cardiomyopathy and a left ventricular ejection fraction <55% have higher urinary concentrations of kidney injury molecule-1 when compared to healthy controls.[16] In adult patients with congestive heart failure it has been demonstrated that kidney injury molecule-1 is superior to other biomarkers for predicting kidney disease progression.[17] Contrary to other previously studied kidney biomarkers, which act as a signal for cellular injury, tissue inhibitor of metalloproteinases-2 and insulin like growth factor binding protein 7 might act as a signal for early cellular stress.[18,19] Chindarkar et al reported a 50th percentile value of [tissue inhibitor of metalloproteinases-2]•[insulin-like growth factor binding protein 7] of 0.32 in at baseline in 378 apparently healthy adults,[10] which is consistent with the median [tissue inhibitor of metalloproteinases-2]•[-insulin-like growth factor binding protein 7] value of 0.28 found in the apparently healthy participants in our study. Their study also showed lower baseline levels of tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 in older subjects when compared to younger participants.[10] In patients without acute kidney injury, Heung and colleagues reported lower tissue inhibitor of metalloproteinases-2 and insulin-like growth factor binding protein 7 values in patients with chronic kidney disease compared to those without chronic kidney disease.[11] Maintaining a constant baseline level of these biomarkers may reflect the adaptive ability of a cell to quickly halt progression through the cell cycle when needed. It is possible that lower levels of insulin-like growth factor binding protein 7 and tissue inhibitor of metalloproteinases-2 are indicative of "frail kidneys" in the patients with congenital heart disease, possibly reflecting less ability to protect themselves from injury.

No patients included in our cohort had evidence of hypoxemia, which can negatively impact renal function, though potentially increased blood viscosity or direct injury.[20,21] Greater levels of cyanosis may result in higher degrees of tubular injury, possibly explaining why we found no differences in the urinary levels of alpha 1-microglobin, beta 2-microglobin, liver fatty acid binding protein, and N-acetyl-B-D-glucosaminidase when comparing subjects with and without congenital heart disease. Prior investigations reporting differences in these tubular biomarkers have included patients with cyanotic heart disease.[5-7]

Given that, this is a small single-center study, there are limitations to the generalizability of our study findings. Although the control group included adults without a known history of cardiac or renal disease, there may be differences in their baseline characteristics. Although there is no evidence that medication use can impact urinary biomarker values, our patient cohort was taking a variety of medications (Table 2) and a medication history was not obtained from the healthy adults. The top four medications reported by our patient cohort (furosemide, aspirin, metoprolol, and lisinopril) have not been shown to have any significant inference with the NephroCheck™ Test when evaluated at multiple drug concentrations.[22] We found similar [tissue inhibitor of metalloproteinases-2]•[insulin-like growth factor binding protein 7] values when comparing patients who reported taking vs not taking the medications.

It is plausible that the differences that we detected in biomarker values are due to low level heart failure in the patients with congenital heart disease. Interestingly, Heung and colleagues report that urinary levels of tissue inhibitor of metalloproteinase-2 and insulin like growth factor binding protein 7 were not impacted by chronic conditions, including congestive heart failure.[11] It would be important in future investigations to collect data on N-terminal pro-brain natriuretic peptide and exercise testing. Additionally, we did not compare differences in markers of glomerular filtration rate, such as serum cystatin C, which has been found to differ in adults with Fontan circulation when compared to controls.[23] Importantly, despite the relatively small sample size of our study we did find significant differences in urinary levels of kidney injury molecule-1, insulin-like growth factor binding protein 7 and tissue inhibitor of

metalloproteinases-2 when comparing individuals of similar age and gender with and without congenital heart disease. How these differences are associated with acute and chronic changes in kidney function after insult remains unknown.

In conclusion, this study shows that early tubular injury may be detected before abnormalities of traditional indicators of kidney dysfunction. The young adult congenital heart disease population is a growing population with an increased risk of kidney disease, but there is no standard method to predict their risk of acute kidney injury or chronic kidney disease. A rise in serum creatinine is seen after kidney injury has occurred. There is a need for research attention on the use of urinary biomarkers to detect deleterious changes in renal function early. Future work identifying associations of biomarker values with renal outcomes in young adults with congenital heart disease could help guide surveillance during routine clinic visits and in their postoperative care.

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CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

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Statistics: Fuhrman
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