Cardiology in the Young

cambridge.org/cty

Evaluation of subclinical cardiovascular risk and cardiac function in children with vesicoureteral reflux: a prospective study

Ahmet Midhat Elmacı[1,2], Hayrullah Alp[3] and Muhammet İrfan Dönmez[4]

[1]Department of Pediatric Nephrology, Dr. Ali Kemal Belviranlı Women's Maternity and Children's Hospital, Konya, Turkey; [2]Guest Faculty Member of KTO, Department of Pediatrics, Karatay University, Konya 42285, Turkey; [3]Department of Pediatric Cardiology, Dr. Ali Kemal Belviranlı Women's Maternity and Children's Hospital, Konya 42285, Turkey and [4]İstanbul University İstanbul Faculty of Medicine, Department of Urology, Division of Pediatric Urology, İstanbul 34093, Turkey

Original Article

Cite this article: Elmacı AM, Alp H, and Dönmez Mİ (2022) Evaluation of subclinical cardiovascular risk and cardiac function in children with vesicoureteral reflux: a prospective study. *Cardiology in the Young* **32**: 1222–1228. doi: 10.1017/S1047951121004005

Received: 24 March 2021
Revised: 12 July 2021
Accepted: 31 August 2021
First published online: 29 September 2021

Keywords:
Vesicoureteral reflux; cardiac functions; Doppler echocardiography; subclinical atherosclerosis; children; renal scar

Author for correspondence:
H. Alp, MD, Assoc. Prof. Department of Pediatric Cardiology, Dr. Ali Kemal Belviranlı Women's Maternity and Children's Hospital, Fatih Mah. Yeni İstanbul Cd. No:32, 42285 Selçuklu, Konya, Turkey. Tel: 0090 506 648 76 53;
Fax: 0090 332 237 60 25.
E-mail: drhayrullahalp@hotmail.com

Abstract

Background: Vesicoureteral reflux is a prominent congenital anomaly of the kidney and the urinary tract. Further, renal scarring is known to be related to chronic inflammation. However, there have been limited studies to date regarding the cardiovascular consequences of vesicoureteral reflux. *Objective:* The aim of this study is to evaluate the possible subclinical atherosclerosis and cardiovascular complications in children with vesicoureteral reflux. *Methods:* Patients with vesicoureteral reflux and age matched healthy controls were prospectively included in this case-control study. Patients were divided into two groups concerning renal scarring status. To assess cardiac functions, carotid artery intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses were evaluated. *Results:* There were 50 patients with vesicoureteral reflux; 26 patients without renal scarring and 24 patients with renal scarring, as well as 40 healthy controls. Myocardial performance indexes (Tei indexes) measured by tissue Doppler echocardiography from septum and left ventricle were significantly increased in study group (for all, $p < 0.001$). Also, intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses of the study groups were significantly higher than the control group (for all, $p < 0.001$). However, no statistical difference was observed between renal scarring (−) and renal scarring (+) groups. *Conclusions:* Results of our study showed early deterioration of cardiac systolic and diastolic functions in children with vesicoureteral reflux regardless of renal scarring. Also, diagnosis of vesicoureteral reflux is an important risk factor for subclinical atherosclerosis, independent of renal scarring, which should be considered in the follow-up of these patients.

Vesicoureteral reflux is one of the most common urological abnormalities in children and it is an important risk factor for recurrent urinary tract infections and subsequent renal scarring.[1] The incidence of vesicoureteral reflux is 1.3% in healthy children and 8–50% in children with urinary tract infections.[2] Also, the incidence of renal scarring has been reported from 36 to 56% in children with urinary tract infections and vesicoureteral reflux.[1] Renal scarring formation is a multifactorial process, and it is the result of an inflammatory response that includes chemotaxis-phagocytosis, lysosomal enzyme release, tubular ischaemia, and reperfusion injury accompanied by immunological mechanisms. Renal scars are histologically characterised by chronic tubulointerstitial inflammation and fibrotic scarring.

There are two main theories in renal scarring development. First, the natural immune system and inflammatory cells are stimulated by the reflux of infected urine into the kidneys. Second, even if the urine is sterile, it increases intrarenal pressure and causes chronic fibrosis. Consequently, renal parenchymal damage can be caused by congenital (dysplasia) or acquired (after pyelonephritis).[1–3] Reflux nephropathy is responsible for 12–21% of all childhood chronic kidney diseases.[2] In previous studies, different cytokine levels in serum and urine and their relation to vesicoureteral reflux and renal scarring have been investigated. The results explained the potential role of the increase in cytokine levels and inflammation in this process in patients with vesicoureteral reflux and renal scarring. In addition, high cytokine levels are also found in these patients, even in the urinary tract infections-free period suggesting that it may be a sign of chronic inflammation.[4–6]

Chronic inflammation is an independent risk factor for subclinical atherosclerosis, which is associated with increased cardiovascular complications. Recent studies have revealed the role of proinflammatory cytokines in the progression of atherosclerosis.[7,8] Currently, common carotid artery intima media thickness, epicardial adipose tissue thickness, and periaortic adipose tissue thickness are frequently used as markers for detecting subclinical atherosclerosis. Using these markers, the presence of subclinical atherosclerosis in different chronic diseases of childhood

has been demonstrated.[9–12] To date, there are no studies on subclinical atherosclerosis in patients with vesicoureteral reflux and renal scarring.

The aim of the study is to evaluate the possible subclinical atherosclerosis and cardiovascular complications in children with vesicoureteral reflux in pre-adolescents when compared to healthy controls. For this purpose, common carotid artery intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses were measured in patients with vesicoureteral reflux and healthy controls, and a detailed cardiac examination was performed in all study population with tissue Doppler echocardiography.

Methods

Study population and laboratory analysis

Fifty vesicoureteral reflux patients (36 girls, 14 boys, mean age 10.2 ± 3.5 years) followed in our paediatric nephrology clinic between November 2019 and May 2020 were included in this prospective study. In this patient group, vesicoureteral reflux was resolved either spontaneously or after surgical intervention. All children were evaluated using urinalysis and urine culture. Exclusion criteria were CHD, chronic kidney disease, hypertension, patients with acute/recent (within 3 months) urinary tract infections, proteinuria and bilateral renal scarring, overweight, obesity, other accompanying urological abnormalities, and other chronic diseases.

The control group consisted of 40 healthy children and adolescents (24 girls, 16 boys, mean age 10.5 ± 2.3 years) with no acute or chronic diseases. In addition, none of them were overweight or obese.

The diagnosis of vesicoureteral reflux was made by voiding cystourethrogram and graded according to the international reflux study grading system.[13] Renal parenchymal scarring was detected by dimercaptosuccinic acid scintigraphy.[14,15] The patients were divided into two subgroups as those without renal scarring (group I) and those with renal scarring (group II). Subclinical atherosclerosis and cardiac changes were investigated by analysing common carotid artery intima media, epicardial adipose tissue, periaortic adipose tissue thicknesses, and cardiac parameters as defined below between these groups and the control group.

Body weight and height were measured according to standard protocols. BMI was calculated by dividing weight (in kilograms) by the square of height (in metres). Blood pressure was measured with the oscillometric method from right arm using automatic blood pressure device. The average of three readings was obtained from a sitting position after 5 minutes of rest with appropriate cuff size. Fasting blood samples were taken in the a.m. and enzymatic measurement was performed using an automatic analyser for serum urea, creatinine, glucose, uric acid, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol. Glomerular filtration rate was calculated using the modified Schwartz formula.[16] Urine analysis and culture were performed.

Echocardiographic measurements

M-mode echocardiographic measurements

Echocardiographic investigations were performed using Philips Affiniti 50 (Philips Healthcare, Eindhoven, Netherlands) with 2.0–4.0 MHz transducers. Echocardiograms were recorded on a hard disc. All measurements were performed according to the American Society of Echocardiography by the same observer blinded to the patients' clinical details.[17] The measurements were obtained during three consecutive cardiac cycles, and the average values were computed. Ejection fraction and fractional shortening of the left ventricle, interventricular septum systolic and diastolic thickness, left ventricular end-systolic and end-diastolic dimensions, and left ventricular posterior wall systolic and diastolic thicknesses were measured from M-mode echocardiographic tracings obtained at midchordal levelling the parasternal long-axis view. The left ventricular mass was estimated using the anatomically validated formula of Devereux and Reichek.[18] The left ventricular mass index was calculated by dividing the left ventricular mass with the height$^{2.7}$.[18]

Pulsed Doppler echocardiographic measurements

Ventricular functions were evaluated using the following pulsed-wave Doppler echocardiographic parameters: early and late mitral/tricuspid diastolic velocities, early velocity/late velocity ratio, and left ventricle/right ventricle ejection times. Standard measurement techniques were used for evaluation.[17] Myocardial performance index (Tei index in tissue Doppler echocardiography) was calculated using the formula, the sum of isovolumetric contraction and relaxation times divided by the ejection time.[19]

Tissue Doppler echocardiographic measurements

Tissue Doppler velocities were obtained from three locations: the sample volume was positioned on the lateral aspect of each atrioventricular valve annulus and basal portion of interventricular septum. Peak early diastolic myocardial, peak atrial systolic, and peak systolic myocardial velocities and time intervals; isovolumetric contraction time, isovolumetric relaxation time, and contraction time were measured by standard technique.[17] Also, e'/a' and E/e' ratios were calculated. The Tei index (myocardial performance index) was calculated as defined above.[19]

Measurement of epicardial adipose tissue thickness

The epicardial adipose tissue was identified as an echo-free space in the pericardial layers on two-dimensional echocardiography, and its thickness was measured perpendicularly on the free wall of the right ventricle at end diastole from the parasternal long-axis views.[20] The mean epicardial adipose tissue thickness was calculated from three consecutive measurements.

Measurement of common carotid artery intima-media thickness

Longitudinal images of the common carotid artery were obtained by combined two-dimensional mode and colour Doppler examinations. A longitudinal echocardiographic image of the posterior wall of the carotid artery was displayed as two bright white lines separated by a hypoechogenic space.[21] The mean common carotid artery intima media thickness was calculated from the three consecutive measurements of the maximum far wall thickness obtained from 10 mm below the carotid bulb.

Measurements of periaortic adipose tissue thickness

Measurement of perivascular adipose tissue was done with conventional methods from the adventitia layer of the abdominal aorta and the adventitial layer of the aorta adjacent to the form of the measurement of the linear echogenic line. Periaortic adipose tissue could no't be distinguished with echocardiographic and ultrasonographic images in deep tissue. Therefore, it was measured within adventitia. Measurements were taken in the axial plane in the supine position at the L1–2 level (just above the umbilicus),

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press

Table 1. Demographic and laboratory data of study population.

	Group I (RS − / n = 26)	Group II (RS +/n = 24)	Controls (n = 40)	p value
Age (years)	10.39 ± 3.62	9.99 ± 3.46	10.50 ± 2.30	0.805
Gender (male/female)	16/10	20/4	24/16	0.128
BMI (kg/m^2)	17.17 ± 2.83	16.91 ± 3.07	17.17 ± 2.33	0.718
SBP (mmHg)	100.38 ± 8.24	100.00 ± 7.22	102.00 ± 9.92	0.782
DBP (mmHg)	63.85 ± 5.71	65.00 ± 6.59	65.50 ± 7.14	0.696
eGFR (ml/min/1.73/m^2)	90.27 ± 12.58	90.13 ± 14.50	90.28 ± 13.23	0.971
Uric acid (mg/dL)	3.92 ± 0.98	3.87 ± 0.98	3.60 ± 0.74	0.269
Cholesterol (mg/dL)	157.38 ± 29.88	156.65 ± 3.75	158.33 ± 28.43	0.900
Triglyceride (mg/dL)	86.79 ± 25.39	101.40 ± 45.54	87.72 ± 35.59	0.463
LDL-C (mg/dL)	84.54 ± 19.54	90.17 ± 22.32	91.58 ± 27.02	0.496
HDL-C (mg/dL)	52.36 ± 13.90	50.66 ± 11.67	46.63 ± 11.69	0.178
Haemoglobin (g/dL)	13.59 ± 1.24	13.79 ± 0.89	13.49 ± 1.06	0.559

Data are expressed mean ± SD
BMI = body mass index; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; RS = renal scarring; SBP = systolic blood pressure; SD = standard deviation

proximal to the iliac bifurcation.[11–22] Evaluation was repeated three times and the mean value was calculated.

Statistical analysis

The data were analysed by statistical computer programme SPSS 25.0 (SPSS Inc., Chicago, IL, United States of America). The compatibility of numerical variables to normal distribution was examined by Shapiro–Wilk test. Descriptive findings were presented as number, percentage mean, and standard deviation. Comparisons between groups were made by Chi-square test for categorical variables, and one-way ANOVA if assumptions were provided for numerical variables, otherwise by Kruskal–Wallis and Mann–Whitney U test. In cases that statistical significance was found in the one-way ANOVA test, the *post hoc* Tukey test was used to identify the group that caused the difference. Where the Kruskal–Wallis test was statistically significant, subgroup analysis was performed using the Mann–Whitney U test. Statistical significance level was accepted as $p < 0.05$.

Results

Patient characteristics

A total of 90 children, including 50 patients and 40 healthy controls, were included in the study. There were 26 patients (16 girls and 10 boys) in group I and 24 patients (20 girls and 4 boys) in group II. There was no statistically significant difference between the patient and control groups in terms of age, gender, body mass index, blood pressure, and biochemical parameters (Table 1).

M-mode and pulsed Doppler echocardiographic data

M-mode and pulsed Doppler echocardiographic measurements revealed no statistical difference between the study groups (Table 2). No significant difference in ventricular mass and left ventricular mass index was found between the groups (group I (without renal scarring): 86.49 ± 36.09 g and 31.53 ± 3.75 g/m$^{2.7}$; group II (with renal scarring): 72.54 ± 24.60 g and 29.85 ± 4.99 g/m$^{2.7}$; control group: 80.55 ± 27.02 g and 30.53 ± 5.16 g/m$^{2.7}$; $p > 0.05$ for all).

Tissue Doppler echocardiographic data

Mitral valve lateral annulus and interventricular septum peak systolic myocardial velocities were significantly higher in groups I (without renal scarring) and II (with renal scarring) (13.07 ± 2.47 and 13.15 ± 2.35, respectively) than the controls (9.43 ± 2.70, $p < 0.001$). However, tricuspid valve lateral annulus peak systolic myocardial velocity was significantly higher in controls ($p = 0.009$) (Table 3). Also, peak early diastolic myocardial and peak atrial systolic velocities measured from both Tissue Doppler echocardiographic regions were significantly lower in group I and group II than the control group ($p < 0.001$ and $p = 0.025$). On the other hand, peak early diastolic myocardial velocity/peak atrial systolic velocity ratios measured from both echocardiographic regions were significantly lower in group I and group II than the controls ($p < 0.001$ and $p = 0.028$). Early diastolic velocity/peak early diastolic velocity ratios calculated from both echocardiographic regions were significantly higher in groups I and II than the control group (for all $p < 0.001$). Among the time intervals, isovolumetric contraction time and isovolumetric relaxation time measured from both mitral and tricuspid lateral annuluses and isovolumetric relaxation time interval of interventricular septum were higher in study groups than the controls (for all $p < 0.05$). Adversely, contraction time measured from both Tissue Doppler echocardiographic regions was significantly lower in group I (without renal scarring) and group II (with renal scarring) than the controls (for all $p < 0.001$).

Additionally, Tei indexes of left ventricle, right ventricle, and interventricular septum in study groups were found to be higher than the control group. However, statistical significance was achieved only for septum Tei index of group I (without renal scarring) and left ventricular Tei index of group II (with renal scarring) (for all $p < 0.001$).

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press

Table 2. M-mode and pulsed Doppler echocardiographic measurements in patients with and without renal scarring and control groups.

	Group I (RS − / n = 26)	Group II (RS +/n = 24)	Controls (n = 40)	p value
M-mode echocardiographic measurements				
IVSd (cm)	0.69 ± 0.12	0.66 ± 0.10	0.69 ± 0.13	0.669
IVSs (cm)	1.15 ± 0.9	1.15 ± 0.20	1.13 ± 0.21	0.887
LVPWd (cm)	0.72 ± 0.10	0.70 ± 0.11	0.71 ± 0.17	0.650
LVPWs (cm)	1.13 ± 0.21	1.12 ± 0.23	1.18 ± 0.18	0.401
LVEdD (cm)	4.09 ± 0.61	3.81 ± 0.42	3.99 ± 0.34	0.091
LVEsD (cm)	2.33 ± 0.43	2.21 ± 0.30	2.38 ± 0.27	0.164
EF (%)	73.63 ± 4.58	73.05 ± 6.38	72.43 ± 9.37	0.555
FS (%)	42.43 ± 3.76	41.79 ± 5.84	40.18 ± 4.89	0.155
LVM (g)	86.49 ± 36.09	72.54 ± 24.60	80.55 ± 27.02	0.365
LVMI ($g/m^{2.7}$)	31.53 ± 3.75	29.85 ± 4.99	30.53 ± 5.16	0.451
Mitral valve blood flow				
Peak E (cm/s)	84.58 ± 9.23	81.88 ± 10.50	86.30 ± 13.96	0.358
Peak A (cm/s)	52.31 ± 11.23	55.31 ± 11.47	57.01 ± 12.30	0.290
E/A ratio	1.68 ± 0.37	1.53 ± 0.31	1.55 ± 0.28	0.271
LV ejection time (ms)	272.92 ± 20.96	273.50 ± 16.87	285.50 ± 27.92	0.052
Tricuspid valve blood flow				
Peak E (cm/s)	62.35 ± 7.19	59.18 ± 8.61	64.33 ± 11.96	0.231
Peak A (cm/s)	46.04 ± 10.70	47.40 ± 12.57	51.18 ± 12.21	0.164
E/A ratio	1.41 ± 0.33	1.30 ± 0.23	1.29 ± 0.22	0.318
RV ejection time (ms)	279.77 ± 25.49	279.21 ± 19.92	281.48 ± 30.09	0.684
LV MPI	0.215 ± 0.113	0.204 ± 0.086	0.164 ± 0.079	0.064
RV MPI	0.164 ± 0.080	0.143 ± 0.078	0.153 ± 0.087	0.562

A = late mitral/tricuspid diastolic velocity; E = early mitral/tricuspid diastolic velocity; EF = ejection fraction; FS = fractional shortening; IVSd = interventricular septum diastolic thickness; IVSs = interventricular septum systolic thickness; LV = left ventricle; LVEdD = left ventricular end-diastolic dimension; LVEsD = left ventricular end-systolic dimension; LVM = left ventricular mass; LVMI = left ventricular mass index; LVPWd = left ventricular posterior wall diastolic thickness; LVPWs = left ventricular posterior wall systolic thickness; MPI = myocardial performance index; RS = renal scarring; RV = right ventricle

Data of common carotid artery intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses

Common carotid artery intima media thickness (0.046 ± 0.012 and 0.047 ± 0.010 cm), epicardial adipose tissue thickness (0.436 ± 0.109 and 0.429 ± 0.098 cm), and periaortic adipose tissue thickness (0.149 ± 0.032 and 0.147 ± 0.022 cm) of the study groups were significantly higher than the control group (for all, $p < 0.001$) (Table 4). However, no statistical difference was observed between group I (without renal scarring) and group II (with renal scarring).

Discussion

Renal cortical abnormalities are frequently encountered in children with vesicoureteral reflux, even without a history of urinary tract infections. However, the relationship between vesicoureteral reflux-associated renal damage and urinary tract infections is well-known.[1] On the other hand; recent studies regarding vesicoureteral reflux and renal scarring show that renal scarring may develop without urinary tract infections. Also, the role of inflammatory cytokines in renal scarring formation has been investigated in many studies.[4,6]

Previous studies on children with vesicoureteral reflux revealed increased levels of proinflammatory markers such as interleukin-6, interleukin-8, and transforming growth factor beta-1 in urine or serum.[4,6,23,24] All these studies show that the inflammation continues in children with vesicoureteral reflux, independent of urinary tract infections and even if vesicoureteral reflux is treated. In our study, common carotid artery intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses were found to be significantly higher in both scarring and non-scarring patients than in the control group. This finding supports that vesicoureteral reflux alone may cause chronic inflammation even if it does not cause scarring.

Persistent elevation of some proinflammatory markers, such as interleukin-6 and interleukin-8, results in a chronic condition defined as subclinical or low-grade inflammation that plays a key role in the development of cardiovascular disease.[7,8,25] In daily practice, common carotid artery intima media thickness is used as a reliable marker in the evaluation of atherosclerotic changes in the early period. In a study evaluating the progression of atherosclerosis, a strong relationship between common carotid artery intima media thickness and interleukin-6 was reported.[8] It has been stated that common carotid artery intima media thickness shows subclinical atherosclerosis in different chronic diseases of

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press

Table 3. Measurements of tissue Doppler echocardiographic parameters in patients with and without renal scarring and control groups.

	Group I (RS − / n = 26)	Group II (RS + / n = 24)	Controls (n = 40)	p value
Mitral valve lateral annulus				
e' (cm/s)	10.22 ± 3.39#	9.32 ± 1.74#	15.68 ± 3.35	<***0.001***
a' (cm/s)	6.89 ± 1.26*	7.01 ± 1.37*	8.29 ± 2.73	***0.025***
s' (cm/s)	13.07 ± 2.47#	13.15 ± 2.35#	9.43 ± 2.70	<***0.001***
IVCT (ms)	56.27 ± 8.95*	53.08 ± 6.67	50.03 ± 9.69	***0.021***
IVRT (ms)	57.04 ± 9.54*	59.54 ± 8.34*	51.20 ± 8.47	***0.002***
CT (ms)	151.31 ± 45.35#	133.00 ± 25.2#	270.03 ± 30.64	<***0.001***
e'/a' ratio	1.48 ± 0.39#	1.38 ± 0.42#	2.00 ± 0.53	<***0.001***
E/e' ratio	9.02 ± 2.55#	9.10 ± 2.12#	5.70 ± 1.28	<***0.001***
Tricuspid valve lateral annulus				
e' (cm/s)	10.96 ± 1.28#	10.66 ± 1.38#	14.84 ± 2.53	<***0.001***
a' (cm/s)	7.80 ± 1.42#	8.43 ± 1.83#	10.11 ± 1.94	<***0.001***
s' (cm/s)	12.55 ± 2.19*	12.48 ± 1.70*	13.87 ± 2.10	***0.009***
IVCT (ms)	54.35 ± 6.64*	50.00 ± 5.82	48.53 ± 7.36	***0.004***
IVRT (ms)	58.96 ± 6.26*	57.50 ± 4.10*	51.90 ± 9.25	***0.001***
CT (ms)	156.69 ± 34.43#	147.67 ± 23.6#	261.78 ± 27.99	<***0.001***
e'/a' ratio	1.40 ± 0.23	1.30 ± 0.24*	1.51 ± 0.33	***0.028***
E/e' ratio	5.76 ± 0.94#	5.61 ± 0.85#	4.40 ± 0.85	<***0.001***
Interventricular septum				
e' (cm/s)	8.32 ± 1.64#	7.36 ± 1.02#	13.40 ± 1.99	<***0.001***
a' (cm/s)	5.68 ± 0.88#	5.65 ± 0.58#	6.79 ± 1.21	<***0.001***
s' (cm/s)	9.99 ± 2.38*	9.96 ± 2.01*	8.63 ± 1.31	***0.004***
IVCT (ms)	52.08 ± 8.07	50.25 ± 5.25	49.00 ± 9.03	0.090
IVRT (ms)	55.88 ± 6.88#	54.63 ± 7.04#	47.35 ± 7.20	<***0.001***
CT (ms)	141.58 ± 43.82#	130.54 ± 27.8#	265.08 ± 22.34	<***0.001***
e'/a' ratio	1.45 ± 0.43#	1.31 ± 0.20#	2.03 ± 0.43	<***0.001***
LV Tei index	0.428 ± 0.153	0.458 ± 0.086#	0.366 ± 0.076	<***0.001***
RV Tei index	0.434 ± 0.176	0.396 ± 0.050	0.387 ± 0.043	0.629
Septum Tei index	0.469 ± 0.194#	0.426 ± 0.057	0.368 ± 0.038	<***0.001***

The p values given on the last column (italic) indicate the statistical difference between three groups
a' = peak atrial systolic myocardial velocities; CT = contraction time; e' = peak early diastolic myocardial velocities; IVCT = isovolumetric contraction time; IVRT = isovolumetric relaxation time; LV = left ventricle; RS = renal scarring; RV = right ventricle; s' = peak systolic myocardial velocities
#p < 0.001 versus control
*p < 0.05 versus control

Table 4. Measurements of cIMT, EFT, and PFT thickness in patients with and without renal scarring and control groups.

	Group I (RS − / n = 26)	Group II (RS + / n = 24)	Controls (n = 40)	p value
cIMT (cm)	0.046 ± 0.012*	0.047 ± 0.010*	0.026 ± 0.003	<***0.001***
EAT thickness (cm)	0.436 ± 0.109*	0.429 ± 0.098*	0.344 ± 0.045	<***0.001***
PAT thickness (cm)	0.149 ± 0.032*	0.147 ± 0.022*	0.115 ± 0.009	<***0.001***

The p values given on the last column (italic) indicate the statistical difference between three groups
cIMT = carotid intima-media thickness; EAT = epicardial adipose tissue; PAT = periaortic adipose tissue; RS = renal scarring
*p < 0.001 versus control

childhood.[10,26] Also, common carotid artery intima media thickness has consistently been related to future cardiovascular disease events in population studies.[27] Common carotid artery intima media thickness is significantly related to other markers for cardiovascular disease risk, such as elevated levels of risk factors and presence of atherosclerosis in the coronary arteries. Furthermore, almost all lipid-lowering trials and a large number of blood pressure lowering trials have consistently shown a

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press

reduction in progression of common carotid artery intima media thickness.[27] In our study, common carotid artery intima media thickness was significantly higher in both renal scarring and non-scarring patient groups when compared to the controls, but no significant difference was found between these two study groups.

On the other hand, body fat distribution is an important cardiovascular risk factor, and fat stores are associated with mortality from all causes. One component of abnormal body fat is the accumulation of adipose tissue around organs and vessels that is called "ectopic fat". Ectopic adipose tissue, unlike subcutaneous adipose tissue, is not a usual place for lipid storage. Epicardial and periaortic adipose tissue also has endocrine functions that can produce inflammatory cytokines and hormones like other adipose tissues. Moreover, they have been lately identified as a powerful risk factor for cardiovascular disease because of their role in the inflammatory process in atherosclerosis.[9,11,20,28] It has been reported that epicardial adipose tissue thickness in adult chronic kidney disease is a reliable parameter in evaluating cardiovascular risk and it predicts coronary artery disease.[28,29] Also, a recent study showed that epicardial fat volume is a significant univariate predictor of the number of coronary plaques and cardiovascular adverse events in asymptomatic adult patients with diabetes.[30] There is a limited number of paediatric studies evaluating epicardial adipose tissue and periaortic adipose tissue thicknesses in obese children with coronary artery disease, increased common carotid artery intima media thickness, and arterial stiffness.[31] Akyürek et al evaluated the relationship between periaortic adipose tissue thickness and cardiovascular risk in 135 children with type-1 diabetes mellitus. In this study, they reported that there is a positive correlation between periaortic adipose tissue and common carotid artery intima media thicknesses and metabolic risk factors.[11] In our study, epicardial adipose tissue and periaortic adipose tissue thickness were found to be significantly higher in both renal scarring and non-scarring patient groups. However, there was no significant difference between renal scarring and non-scarring patient groups.

It is known that E/e' ratio shows the strongest correlation with left ventricular/right ventricular diastolic filling pressure and left ventricle/right ventricle compliance,[32,33] whereas early diastolic velocity/late diastolic velocity and peak early diastolic myocardial velocity/peak atrial systolic myocardial velocity ratios correlate with relaxation-type dysfunction.[34] Limited data from the children with chronic kidney diseases revealed that left ventricular early diastolic velocity/late diastolic velocity and peak early diastolic myocardial velocity/peak atrial systolic myocardial velocity ratios decrease and early diastolic velocity/peak early diastolic myocardial velocity ratio increases along with the worsening of renal functions from mild–moderate to severe renal failure.[35] On the other hand, Çelik et al reported decreased early diastolic velocity/late diastolic velocity and increased peak early diastolic myocardial velocity/peak atrial systolic myocardial velocity ratios in non-obese-treated hypertensive patients.[35] These studies showed dysfunction of left ventricular relaxation and diastolic filling pressures; however, right ventricular functions were not studied and possibly right ventricular dysfunction was underestimated. In this context, our study revealed that e'/a' ratios measured from left ventricle, right ventricle, and interventricular septum were significantly lower in renal scarring (+) (group II) and scarring (–) (group I) vesicoureteral reflux patients when compared with healthy controls. Additionally, early diastolic velocity/peak early diastolic myocardial velocity ratios measured from left ventricle and right ventricle were detected to be significantly increased in both patient groups. These findings suggest that left and right ventricle functions and diastolic filling pressures are worsened in children with vesicoureteral reflux regardless of renal scarring formation.

On the other hand, myocardial performance index or Tei index is a good predictor of ventricular systolic functions in children and adults.[19] Myocardial performance index measured by pulsed-wave Doppler, M-mode, and tissue Doppler imaging methods is a valuable parameter indicating systolic and diastolic functions. The results from the previously mentioned study showed that left ventricular myocardial performance index was higher in non-obese-treated hypertensive children, but no significance was achieved.[35] However, in our study myocardial performance index values of left and right ventricles in the group without renal scarring were significantly higher than the controls while no statistical significance was shown. Besides, both Tei index values of left ventricle, right ventricle, and interventricular septum were found to be increased in the both study groups than in the controls. Nevertheless, statistical significance was achieved for only left ventricular Tei index of renal scarring (+) (group II) and septum Tei index of renal scarring (–) (group I) groups. By this way, we demonstrated a significant reduction of systolic and diastolic functions of left and right ventricles in children with vesicoureteral reflux regardless of renal scarring formation compared to healthy children.

It is important to consider the limitations pertaining to the methods of this study. The main limitation of our study is that inflammatory cytokines were not studied. Additionally, the duration of illness (vesicoureteral reflux) and associated factors could not be studied. However, serum lipid parameters as well as a detailed echocardiographic assessment was performed.

Our study showed early deterioration of cardiac systolic and diastolic functions in children with vesicoureteral reflux regardless of renal scarring. Also, common carotid artery intima media, epicardial adipose tissue, and periaortic adipose tissue thicknesses, indicating the presence of early subclinical atherosclerosis, were found to be higher in both renal scarring and non-scarring vesicoureteral reflux patients. These results suggest that vesicoureteral reflux alone is an important risk factor for subclinical atherosclerosis, independent of renal scarring, and should be considered in the follow-up of these patients.

Acknowledgements. None declared.

Financial support. This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Conflicts of interest. None.

Ethical standards. Ethical clearance was sought and obtained from KTO Karatay University, Medical Faculty Ethical Committee (IRB Number: 419011325-050.99). Patients of the study were asked to provide informed voluntary written consent. Assent was also signed by parents for patients under the age of 18 years to be included in the study.

Author contributions. A.M.E conceptualised and designed the study, carried out the initial analyses, drafted the initial manuscript, and reviewed and revised the manuscript. H.A. designed the data collection instruments, collected data, and reviewed and revised the manuscript. M.İ.D. conceptualised and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press

References

1. Mattoo TK. Vesicoureteral reflux and reflux nephropathy. Adv Chronic Kidney Dis 2011; 18: 348–354. DOI 10.1053/j.ackd.2011.07.006.
2. Mattoo TK, Mathews R. Vesicoureteral reflux and renal scarring. In: Avner ED, Harman WE, Niaudet P, Yoshikawa N (eds). Pediatric Nephrology. Springer, Berlin, 2009: 1311–1328.
3. Fillion ML, Watt CL, Gupta IR. Vesicoureteric reflux and reflux nephropathy: from mouse models to childhood disease. Pediatr Nephrol 2014; 29: 757–766. DOI 10.1007/s00467-014-2761-3.
4. Gokce I, Alpay H, Biyikli N, Unluguzel G, Dede F, Topuzoglu A. Urinary levels of interleukin-6 and interleukin-8 in patients with vesicoureteral reflux and renal parenchymal scar. Pediatr Nephrol 2010; 25: 905–912. DOI 10.1007/s00467-009-1396-2.
5. Galanakis E, Bitsori M, Dimitriou H, Giannakopoulou C, Karkavitsas NS, Kalmanti M. Urine interleukin-8 as a marker of vesicoureteral reflux in infants. Pediatrics 2006; 117: e863–867. DOI 10.1542/peds.2005-2051.
6. Sabasińska A, Zoch-Zwierz W, Wasilewska A, Porowski T. Laminin and transforming growth factor beta-1 in children with vesicoureteric reflux. Pediatr Nephrol 2008; 23: 769–774. DOI 10.1007/s00467-007-0723-8.
7. Moreira DM, da Silva RL, Vieira JL, Fattah T, Lueneberg ME, Gottschall CA. Role of vascular inflammation in coronary artery disease: potential of anti-inflammatory drugs in the prevention of atherothrombosis. Inflammation and anti-inflammatory drugs in coronary artery disease. Am J Cardiovasc Drugs 2015; 15: 1–11. DOI 10.1007/s40256-014-0094-z.
8. Okazaki S, Sakaguchi M, Miwa K, Furukado S, Yamagami H, Yagita Y. Association of interleukin-6 with the progression of carotid atherosclerosis: a 9-year follow-up study. Stroke 2014; 45: 2924–2929. DOI 10.1161/STROKEAHA.114.005991.
9. Djaberi R, Schuijf JD, van Werkhoven JM, Nucifora G, Jukema JW, Bax JJ. Relation of epicardial adipose tissue to coronary atherosclerosis. Am J Cardiol 2008; 102: 1602–1607. DOI 10.1016/j.amjcard.2008.08.010.
10. Bakkaloglu SA, Saygili A, Sever L, et al. Assessment of cardiovascular risk in paediatric peritoneal dialysis patients: a Turkish Pediatric Peritoneal Dialysis Study Group (TUPEPD) report. Nephrol Dial Transplant 2009; 24: 3525–3532. DOI 10.1093/ndt/gfp297.
11. Akyürek N, Atabek ME, Eklioglu BS, Alp H. Evaluation of the relationship between cardiovascular risk factors and periaortic fat thickness in children with type 1 diabetes mellitus. Diabetes Metab 2015; 41: 338–341. DOI 10.1016/j.diabet.2015.02.005.
12. de Ferranti SD, Steinberger J, Ameduri R, et al. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association. Circulation 2019; 139: e603–e634. DOI 10.1161/CIR.0000000000000618.
13. Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen-Möbius TE. International system of radiographic grading of vesicoureteric reflux. International reflux study in children. Pediatr Radiol 1985; 15: 105–109.
14. Imperiale A, Olianti C, Sestini S, et al. 123I-hippuran renal scintigraphy with evaluation of single-kidney clearance for predicting renal scarring after acute urinary tract infection: comparison with (99m)Tc-DMSA scanning. J Nucl Med 2003; 44: 1755–1760.
15. Cain JE, Di Giovanni V, Smeeton J, Rosenblum ND. Genetics of renal hypoplasia: insights into the mechanisms controlling nephron endowment. Pediatr Res 2010; 68: 91–98. DOI 10.1203/PDR.0b013e3181e35a88.
16. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009; 20: 629–637. DOI 10.1681/ASN.2008030287.
17. Lang RM, Bierig M, Devereux RB, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18: 1440–1463. DOI 10.1016/j.echo.2005.10.005.
18. Devereux RB, Reichek N. Echocardiographic determination of left ventricularmass in man. Anatomic validation of the method. Circulation 1977; 55: 613–618.
19. Tei C. New non-invasive index for combined systolic and diastolic ventricular function. J Cardiol 1995; 26: 135–136.
20. Iacobellis G, Bianco AC. Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. Trends Endocrinol Metab 2011; 22: 450–457. DOI 10.1016/j.tem.2011.07.003.
21. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. Cerebrovasc Dis 2012; 34: 290–296. DOI 10.1159/000343145.
22. Akyürek N, Atabek ME, Eklioglu BS, Alp H. The relationship of periaortic fat thickness and cardiovascular risk factors in children with Turner syndrome. Pediatr Cardiol 2015; 36: 925–929. DOI 10.1007/s00246-015-1098-4.
23. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. N Engl J Med 1994; 331: 1286–1292.
24. Morozova O, Morozov D, Pervouchine D, et al. Urinary biomarkers of latent inflammation and fibrosis in children with vesicoureteral reflux. Int Urol Nephrol 2020; 52: 603–610. DOI 10.1007/s11255-019-02357-1.
25. Marchio P, Guerra-Ojeda S, Vila JM, Aldasoro M, Victor VM, Mauricio MD. Targeting early atherosclerosis: a focus on oxidative stress and inflammation. Oxid Med Cell Longev 2019; 2019: 8563845. DOI 10.1155/2019/8563845.
26. Järvisalo MJ, Jartti L, Näntö-Salonen K, et al. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation 2001; 104: 2943–2947. DOI 10.1161/hc4901.100522.
27. Bots ML. Carotid intima-media thickness as a surrogate marker for cardiovascular disease in intervention studies. Curr Med Res Opin 2006; 22: 2181–2190. DOI 10.1185/030079906X148472.
28. Aeddula NR, Cheungpasitporn W, Thongprayoon C, Pathireddy S. Epicardial adipose tissue and renal disease. J Clin Med 2019; 8: 299. DOI 10.3390/jcm8030299.
29. Nakanishi K, Fukuda S, Tanaka A, et al. Epicardial adipose tissue accumulation is associated with renal dysfunction and coronary plaque morphology on multidetector computed tomography. Circ J 2016; 80: 196–201. DOI 10.1253/circj.CJ-15-0477.
30. Venuraju SM, Lahiri A, Jeevarethinam A, Rakhit RD, Shah PK, Nilsson J. Association of epicardial fat volume with the extent of coronary atherosclerosis and cardiovascular adverse events in asymptomatic patients with diabetes. Angiology 2021; 72: 442–450. DOI 10.1177/0003319720984607.
31. Manco M, Morandi A, Marigliano M, Rigotti F, Manfredi R, Maffeis C. Epicardial fat, abdominal adiposity and insulin resistance in obese prepubertal and early pubertal children. Atherosclerosis 2013; 226: 490–495. DOI 10.1016/j.atherosclerosis.2012.11.023.
32. Harada K, Tamura M, Yasuoka K, Toyono M. A comparison of tissue Doppler imaging and velocities of transmitral flow in children with elevated left ventricular preload. Cardiol Young 2001; 11: 261–268. DOI 10.1017/s1047951101000270.
33. Lopez L, Colan SD, Frommelt PC, et al. Recommendations for quantification methods during the performance of a pediatric echocardiogram: a report from the Pediatric Measurements Writing Group of the American Society of Echocardiography Pediatric and Congenital Heart Disease Council. J Am Soc Echocardiogr 2010; 23: 465–495. DOI 10.1016/j.echo.2010.03.019.
34. Doyon A, Haas P, Erdem S, et al. Impaired systolic and diastolic left ventricular function in children with chronic kidney disease - results from the 4C study. Sci Rep 2019; 9: 11462. DOI 10.1038/s41598-019-46653-3.
35. Celik SF, Karakurt C, Tabel Y, Elmas T, Yologlu S. Blood pressure is normal, but is the heart? Pediatr Nephrol 2018; 33: 1585–1591. DOI 10.1007/s00467-018-3968-5.

https://doi.org/10.1017/S1047951121004005 Published online by Cambridge University Press