Abnormal Carotid Artery Structure and Function in Children and Adolescents With Successful Renal Transplantation

Mark M. Mitsnefes, MD, MS; Thomas R. Kimball, MD; Sandra A. Witt, RDCS; Betty J. Glascock, RDCS; Philip R. Khoury, MS; Stephen R. Daniels, MD, PhD

Background—Abnormal carotid artery compliance and increased intima-media thickness (IMT), markers of early atherosclerosis, are prevalent in adults with chronic kidney failure. However, little is known about the extent of these abnormalities in children after transplantation.

Methods and Results—Thirty-one children (age, 14.5±4.1 years) with renal transplant (estimated glomerular filtration rate, 78.1±24.5 mL/min per 1.73 m²; range, 44 to 128 mL/min per 1.73 m²) and 33 age- and sex-matched control subjects had ultrasound of the carotid artery, echocardiography, and ambulatory blood pressure monitoring (transplant patients only). IMT was measured, and distensibility and stiffness parameter (β) were calculated to assess carotid artery structure and function. The results were correlated with demographic, clinical, and biochemical variables. Compared with control subjects, children with transplant had higher IMT (P<0.03) and lower distensibility (P<0.001). In multiple regression analysis, increased IMT in children who had received transplants was associated with higher mean office systolic blood pressure taken within 1 year before the study (R²=0.19, P=0.024) and receipt of >1 transplant (R²=0.16, P=0.02). Worse distensibility and β were significantly associated with higher daytime systolic blood pressure load calculated from ambulatory blood pressure and receipt of cadaveric kidney. When number of antihypertensives was added to the models, only higher number of blood pressure medications independently predicted abnormal distensibility (R²=0.38, P=0.002) and β (R²=0.25, P=0.016).

Conclusions—Carotid arteriopathy is present in children with successful renal transplant and is associated with hypertension. The results suggest that these children might be at risk for accelerated atherosclerosis and premature cardiovascular disease. (Circulation. 2004;110:97-101.)

Key Words: carotid arteries ■ cardiovascular diseases ■ kidney ■ pediatrics ■ transplantation

Cardiovascular disease is a main cause of death in children and young adults after renal transplantation, accounting for up to 40% of all deaths.1,2 It has been suggested that the reason for cardiovascular disease is accelerated atherosclerosis and coronary artery disease. Recent studies in adults who developed end-stage renal disease (ESRD) during childhood found a high prevalence of asymptomatic atherosclerosis as demonstrated by abnormal intima-media thickness (IMT) and arterial wall compliance.3,4 However, from those studies, it is not possible to determine when arterial abnormalities develop.

Pediatric pathology studies have determined that the atherogenic process might well begin in childhood and that atherosclerotic lesions are associated with hyperlipidemia and hypertension.5 These risk factors are strongly present in pediatric renal transplant recipients, suggesting that the development of progressive atherosclerosis might have already begun. To test this hypothesis, we designed a study to assess carotid artery structure and arterial wall compliance in children and adolescents after transplantation.

Methods

Patient Characteristics

Inclusion criteria were as follows: 6 to 21 years of age, absence of primary myocardial disease, functioning graft for ≥1 year with estimated glomerular filtration rate (eGFR) ≥40 mL/min per 1.73 m². Of the 65 patients in the transplant clinic, 6 were <6 years of age, 5 had low eGFR, 1 had cardiac anomaly, and 13 had follow-up <1 year. From 40 eligible subjects, 33 agreed to participate in the study. Two subjects were excluded because of poor ultrasound images. Control subjects were recruited from the families of personnel at Children’s Hospital and siblings of transplant recipients. Thirty-one children with transplant and 33 age- and sex-matched control subjects were included in the final analysis. The Institutional Review Board of the Children’s Hospital Medical Center in Cincinnati (Ohio) approved the study; informed consent was obtained from each subject.

Demographic and clinical information was collected on the day of echocardiography. All patients received triple immunosuppression including steroids (all), calcineurin inhibitors (cyclosporine, n=9; prograf, n=22), and azathioprine (n=5) or mycophenolate mofetil (n=26). Each patient had serum creatinine, calcium, phosphorus, intact parathyroid hormone, hematocrit, fasting lipid profile, homo-
cysteine, and high-sensitivity C-reactive protein (hsCRP) determined. Hyperalbuminemia was defined on the basis of the KDOQI guidelines. Hyperhomocysteinemia was defined as serum homocysteine level >15 μmol/L; hsCRP >0.2 mg/dL (increased cardiac risk in adults) was considered elevated.

Systolic (SBP) and diastolic (DBP) blood pressures were measured at the time of echocardiography by auscultation with a mercury or aneroid sphygmomanometer with the patient in the sitting position and an appropriately sized cuff. The mean values for SBP (1-year SBP) and DBP (1-year DBP) were calculated for BP taken during the 1 year preceding the study. These BP were recorded by 2 trained nurses using standard protocol during the routine clinic visits. There was an average of 11 ± 5 measurements. To control for the differences in age and body size, BP was indexed. Measured BP value was divided by age-, sex-, and height-specific 95th BP percentiles. Hypertension was defined as indexed SBP or DBP ≥1.0.

Ambulatory blood pressure monitoring (ABPM) was performed in transplant recipients with the 90217 model from SpaceLab Medical, Inc. Measurements were taken every 20 minutes. Sleep hours were determined by patient self-report. Mean SBP and DBP were determined for daytime, sleep, and the full 24 hours; indexed BP was calculated. Dipping was defined as a 10% drop in mean BP between daytime and sleep, and nondipping was defined as a decline of <10%. Hypertension was defined as 24-hour, daytime, and nighttime mean SBP and DBP above the 95th percentile. BP load was defined as percentage of BP readings that exceed the 95th percentile.

Cardiac structure and function, including left ventricular (LV) mass index, relative wall thickness, and LV contractility calculation, were determined as described elsewhere.

Carotid artery ultrasound was performed with a GE Vivid 7 Horten Norway, M12L, 5.0- to 11.0-MHz probe. Measurements were obtained with subjects in the supine position by 2 experienced sonographers blinded to the subjects’ clinical status. An ultrasound imager distal to the carotid artery bifurcation on a segment of ≥1-cm length of the posterior wall was used for the study. Measurements were performed for ≥3 consecutive heartbeats. IMT and the internal diameter of the right common carotid artery were then calculated. The intraobserver variability was 1.1% for IMT and 0.0% for arterial wall diameter measurements. Distensibility (DC) was calculated with this formula: DC = 2 × (D/D) / (SBP − DBP), where D is carotid artery diastolic diameter and ΔD is change in artery diameter during systole. The stiffness parameter (β) was calculated from this formula: β = (ln(SBP/DBP)) / (ΔD/D).

Statistical Analysis

Values are presented as mean±SD. A 2-sample t test or the Mann-Whitney rank-sum test was used to compare continuous variables, and the χ² test was used to compare categorical variables. Associations between variables were assessed by Spearman analysis. Stepwise regression analysis was performed to assess independent predictors of abnormal IMT or arterial compliance. Variables with values of P < 0.15 from correlation analyses were entered into the regression analysis. The SAS 8.0 statistical package was used in the analysis. A value of P < 0.05 was considered statistically significant.

Results

Patient Characteristics

Seventeen children (55%) received transplants as a result of ESRD caused by dysplasia/obstructive uropathy and 14 (45%) with glomerular or cystic disease. The median time after transplantation was 3.7 years (range, 1 to 14.3 years). Most of the patients (81%) had their first transplant and were treated by chronic dialysis (68%) before transplantation. The median cumulative duration on dialysis was 1.2 years (range, 0 to 10 years); the median cumulative duration of ESRD (dialysis plus duration of chronic kidney failure, defined as eGFR <15 mL/min per 1.73 m²) was 1.3 years (range, 0.25 to 10.3 years); and the median duration of renal replacement therapy (dialysis plus transplant) was 5.2 years (range, 1.3 to 14.9 years). Sixteen patients (52%) had an allograft from live donors. Five children had prior treated acute rejection. The average eGFR at the time of the study was 78.1 ± 24.5 mL/min per 1.73 m² (range, 44 to 128 mL/min per 1.73 m²). Ten children (32%) had elevated total cholesterol; 7 (23%) had elevated LDL cholesterol; and 19 (62%) had hypertriacylglyceridemia. Only 2 children had calcium-phosphorus product >45 mg²/dL². Hyperhomocysteinemia was found in 7 children (23%), and hsCRP >0.2 mg/dL was found in 5 (16%). There was no significant difference in age or body size between allograft recipients and control subjects (Table 1).

BP Evaluation

Children with transplant had higher casual BP recorded on the day of the study than control subjects. Thirteen transplant recipients (42%) had systolic and 5 (16%) had diastolic hypertension. BP obtained during the year before the study (1-year indexed SBP, 0.96 ± 0.09; 1-year indexed DBP, 0.84 ± 0.12) was similar to casual BP recorded on the day of the study (P = NS). Twenty-three children (74%) were taking antihypertensives: 12 were taking 1 medication, 8 were taking 2 medications, and 3 were taking 3 medications. Nineteen patients were taking calcium channel blockers. Other medications included β-blockers (n = 2), diuretics (n = 3), and ACE inhibitors (n = 7). ABPM demonstrated a high prevalence of daytime and nighttime hypertension, abnormally high BP load, and nondipping status in the transplant recipients (Table 2).

Carotid Artery Evaluation

Children with transplant had higher IMT (0.42 ± 0.07 versus 0.38 ± 0.06 mm; P = 0.03) and stiffness parameter (6.98 ± 3.26 versus 5.30 ± 2.71; P = 0.0001) and significantly lower distensibility (37.4 ± 16.6 versus 53.4 ± 17.3 kPa⁻¹ × 10⁻³; P = 0.0001) compared with control subjects.

In the control subjects, there were no significant independent predictors for carotid IMT. Distensibility could be predicted from pulse pressure (β = 1.21 × 10⁻⁵; R² = 0.61; P = 0.0001) and weight (β = 3.21 × 10⁻⁵; R² = 0.15; P = 0.004); stiffness parameter could be predicted from weight (β = 0.097; R² = 0.23; P < 0.0001) and DBP (β = −0.15; R² = 0.20; P = 0.01).

In children with transplants, IMT was correlated with 1-year SBP (r = 0.38, P = 0.05), LV contractility (r = 0.41,
TABLE 2. ABPM Data (Renal Transplant Subjects)

<table>
<thead>
<tr>
<th>Variable</th>
<th>SBP, %</th>
<th>DBP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h hypertension</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>24-h BP load</td>
<td>43±29</td>
<td>43±29</td>
</tr>
<tr>
<td>24-h load &gt;50%</td>
<td>43</td>
<td>36</td>
</tr>
<tr>
<td>Daytime hypertension</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>Daytime BP load</td>
<td>36±30</td>
<td>34±28</td>
</tr>
<tr>
<td>Daytime load &gt;50%</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>Nighttime hypertension</td>
<td>68</td>
<td>71</td>
</tr>
<tr>
<td>Nighttime BP load</td>
<td>54±36</td>
<td>57±36</td>
</tr>
<tr>
<td>Nighttime load &gt;50%</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>Mean BP dipping</td>
<td>5.6±6.8</td>
<td>8.7±11.0</td>
</tr>
<tr>
<td>Abnormal BP dipping</td>
<td>82</td>
<td>50</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or percentage when indicated.

$P=0.03$, relative wall thickness ($r=0.38$, $P=0.05$), and number of transplants ($r=0.44$, $P=0.03$). Children with multiple transplants were on dialysis longer (median, 3.8 versus 0.7 years; $P<0.001$) and had longer cumulative duration of ESRD (median, 5.2 versus 1.5 years; $P<0.001$) than children with a single transplantation. There was no significant difference between these subgroups in regard to BP control, current immunosuppression therapy, and graft function.

Distensibility and stiffness were significantly correlated with daytime BP from ABPM and with the number of BP medications (Table 3). There was also a significant interaction between arterial wall compliance and donor type (for distensibility: $r=0.45$, $P=0.02$; for $\beta$: $r=0.44$, $P=0.02$). One-way ANOVA was performed to compare the control group and children with living and cadaveric donors. As shown in Table 4, children with cadaveric donors had lower distensibility and higher stiffness than control subjects or children with living donors. IMT was higher in children with a cadaveric donor than in control subjects but was similar to that in children with living donors. There was no significant difference in carotid IMT, distensibility, or stiffness between control subjects and children with living donors.

In transplant patients, no significant associations for IMT, distensibility, and $\beta$ were found with age, weight, height, body mass index, LV mass index, cause of kidney failure, time after transplantation, duration of dialysis treatment or ESRD, current immunosuppression therapy, or any laboratory parameters.

Results of the stepwise multiple regression analysis are presented in Table 5. Increased IMT was associated with higher 1-year SBP and receiving >1 transplant. Worse distensibility and stiffness were associated with higher daytime SBP load and receiving a cadaveric kidney. When number of antihypertensives was added to the same regression models, no significant change in the association with IMT was noted, whereas only higher number of BP medications was independently associated with lower distensibility ($\beta=-2.5\times10^{-3}$; $R^2=0.38$; $P=0.002$) and increased stiffness ($\beta=2.3$; $R^2=0.25$; $P=0.016$). There was no remaining influence of donor type and daytime SBP load.

**Discussion**

The important observation of this study is that children and adolescents with stable renal transplant have impaired carotid artery structure and function as evidenced by increased IMT, abnormally low distensibility, and elevated stiffness. These findings are significant because abnormalities of the carotid artery have been accepted as markers of the early, asymptomatic phase of the atherosclerotic process.

Carotid arteriopathy has been reported in pediatric patients with diabetes and essential hypertension in adults after transplantation. Clinical studies have shown that increased IMT and stiffness of the large arteries independently predict cardiovascular morbidity and mortality in adults with ESRD. Recently, these abnormalities have been demonstrated in young adults who developed ESRD during childhood. For example, Oh et al. found an increase in carotid IMT (mean age, 27 years), whereas Groothoff et al. found increased stiffness and decreased distensibility (mean age, 29 years). In our study, similar abnormalities were found to be present in patients of much younger age (mean age, 14.5 years; range, 6 to 20 years) and good allograft function (mean eGFR, 78±24.5 mL/min per 1.73 m$^2$). These findings are worrisome and indicate that even children and adolescents with successful transplantation might be at increased risk for cardiovascular morbidity.

In the present study, we found that carotid artery changes were significantly related to BP. IMT was significantly associated with SBP recorded over 1-year period before the study, and distensibility and stiffness were associated with BP taken at the day of the study. These data demonstrate that chronic elevation of BP might reflect structural changes (increased IMT), whereas current BP status might reflect functional changes (decreased distensibility and increased stiffness) in the carotid artery.

As in other pediatric studies, we found a high prevalence of abnormal BP detected by ABPM. The important observation in this study is that daytime SBP load was superior in predicting carotid artery compliance than casual BP. In children, the BP load is a percentage of BP reading above the 95th percentile. Sorof et al. have shown that SBP load >50% is associated with LV hypertrophy in children with essential hypertension. Our study demonstrates that, in addition to predicting cardiac hypertrophy, BP load might also be useful for predicting decreased vascular compliance.

**TABLE 3. Correlations Between Indexes of Carotid Wall Compliance and BP Characteristics in Children With Renal Transplant**

<table>
<thead>
<tr>
<th>Variable</th>
<th>DC Regression Coefficient</th>
<th>$\beta$ Regression Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime indexed SBP</td>
<td>-0.42*</td>
<td>0.38*</td>
</tr>
<tr>
<td>Daytime SBP load</td>
<td>-0.49*</td>
<td>0.48*</td>
</tr>
<tr>
<td>Daytime DBP load</td>
<td>-0.42*</td>
<td>0.44*</td>
</tr>
<tr>
<td>No. of BP medications</td>
<td>-0.57*</td>
<td>0.55*</td>
</tr>
</tbody>
</table>

DC indicates distensibility.

* $P<0.05$. 
Despite the high prevalence of nighttime hypertension, we found that daytime but not nighttime BP was significantly associated with carotid artery wall compliance. One of the reasons for this association may be that the measurements of carotid artery were performed during the day. In addition, BP taken during the carotid artery ultrasound is included in the formula to calculate distensibility. Therefore, it is not surprising that distensibility was associated with daytime BP in both control children and children with transplant. Unlike distensibility, stiffness parameter is considered a more reliable measure of arterial compliance, which is independent of BP in healthy adults. In our control subjects, increased stiffness parameter was associated with lower DBP. In contrast, in children after transplantation, increased SBP but not DBP was associated with arterial stiffness. These findings confirm the importance of systolic hypertension in the development of end-organ damage in pediatric renal transplantation recipients.

We also found that carotid IMT was increased in children with multiple transplants. In patients with numerous transplants, chronic exposure to volume and flow/pressure over-load while on dialysis or during periods of significant graft dysfunction creates conditions for arterial remodeling, with the ultimate development of arterial intima-media hypertrophy. Children with multiple transplants had longer duration of ESRD and chronic dialysis treatment compared with patients with a single transplant, but unlike the adult studies, the cumulative duration of dialysis did not predict abnormal arterial IMT or compliance in our study. However, it is important to note that the median cumulative duration of dialysis in our patients (1.2 years) was significantly lower than in the studies by Oh et al (5 years) and Groothof et al (4.5 years).

In the present study, increased IMT was significantly associated with cardiac abnormalities such as increased wall thickness and LV contractility. The interrelationships between cardiac and vascular hypertrophy have been described in adults with ESRD and suggest that similar mechanisms, including pressure and volume overload, might be involved in the development of these abnormalities. Studies of adults also suggest that chronic arterial changes might result in increased LV systolic wall stress and increased cardiac output to satisfy oxygen and metabolic demand of the peripheral tissues.

Arterial stiffness and distensibility were significantly worse in children with cadaveric donors compared with children with living donors. The most likely cause of this difference is more severe hypertension in patients with a cadaveric donor. Among 12 children with a cadaveric kidney, 7 (58%) required ≥2 BP medications, whereas among 19 children with living donors, only 2 (10%) needed ≥1 BP medication. This probably explains why adding number of BP medications to the regression model eliminated donor type as an independent predictor of carotid artery wall compliance.

Studies of adults have shown that hyperlipidemia, hyperhomocysteinemia, hyperparathyroidism, and increased calcium-phosphorus product are associated with increased IMT or abnormal arterial wall compliance after transplantation.
tion.23–25 In contrast, we failed to show significant relationships between carotid artery abnormalities and those markers. However, the subjects of our study were healthier (younger and with better allograft function) and had significantly shorter duration of kidney failure (5.2 years) than reported in the above studies (18 to 19 years). Thus, the exposure to cardiovascular risk factors in our patients was significantly lower compared with adults with ESRD. Recent studies26,27 linked cardiac risk factors during childhood to carotid artery abnormalities in young adults in the general population. Long-term studies assessing the effect of cardiac risk factors on carotid artery compliance in children with renal transplants are necessary.

Study Limitations
From this relatively small, cross-sectional study, it was impossible to determine when the abnormalities of carotid artery develop and how they progress during chronic kidney failure. Some BP data were collected retrospectively. Longitudinal and larger studies are needed to evaluate temporal evolution of these vascular abnormalities and cardiac risk factors in children through different stages of chronic kidney disease. We did not examine patients for insulin resistance, which is a known risk factor for carotid artery abnormalities. In our control group, ABPM was not performed. We also were unable to determine any significant correlates for IMT in this group. However, our data are in agreement with the only large pediatric study by Saas et al.,28 who showed that age, BP, and body size had little influence on carotid IMT. Because there are no established norms for IMT, distensibility, and stiffness parameter in the pediatric population, we were unable to compare transplant patients with and without abnormal carotid artery structure and function.

Conclusions
The present study demonstrates that early atherosclerosis is already present in children and adolescents with successful renal transplantation. Successful transplantation is the ultimate and best available treatment for these patients. Nevertheless, our data suggest that these young patients might be at higher risk for premature cardiovascular morbidity and mortality and support the need for more aggressive management of hypertension to prevent the development and progression of vascular abnormalities in children with chronic kidney disease.

Acknowledgments
This work was supported by grants from the National Institutes of Health (K23 HL-69296-01) and the American Heart Association (grant 0160214B)

References
Abnormal Carotid Artery Structure and Function in Children and Adolescents With Successful Renal Transplantation
Mark M. Mitsnefes, Thomas R. Kimball, Sandra A. Witt, Betty J. Glascock, Philip R. Khoury and Stephen R. Daniels

Circulation. 2004;110:97-101; originally published online June 21, 2004;
doi: 10.1161/01.CIR.0000133412.53089.26
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/1/97

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/