Capillary rarefaction: an early marker of microvascular disease in young hemodialysis patients

Alcia Edwards-Richards1, Marissa DeFreitas1, Chryso P. Katsoufis1, Wacharee Seeherunvong1, Nao Sasaki2, Michael Freundlich1, Gaston Zilleruelo1 and Carolyn L. Abitbol1

1Division of Pediatric Nephrology, University of Miami/Holtz Children’s Hospital, Miami, FL, USA and 2Division of Pediatric Cardiology, University of Miami/Holtz Children’s Hospital, Miami, FL, USA

Correspondence and offprint requests to: Carolyn L. Abitbol; E-mail: cabitbol@med.miami.edu

Abstract

Background. Pediatric patients with chronic kidney disease (CKD) are at increased risk of early cardiovascular disease and premature death. Abnormalities in microvascular structure and function may presage end-organ damage including vascular calcification and myocardial ischemia associated with disordered mineral metabolism. Early detection of microvascular rarefaction (reduced density of capillaries) may identify at-risk patients and prompt timely therapeutic interventions. Our objective was to study capillary rarefaction in pediatric hemodialysis (HD) patients and to determine possible associations with mineral metabolism and cardiac risk biomarkers.

Methods. Capillary density (CD) was measured by nailfold capillaroscopy in 19 pediatric HD patients and 20 healthy controls. Demographic and biochemical markers were collected at entry and 6-month follow-up.

Results. CD was significantly decreased in HD patients compared with controls with a deficit of 24 and 31% at baseline and subsequent follow-up. Maximal CD correlated significantly with intact parathyroid hormone (iPTH) \( r = -0.45; P = 0.005 \), serum calcium \( r = -0.38; P = 0.02 \) and 25(OH) vitamin D levels \( r = +0.36; P = 0.03 \) in HD patients. Capillary functional measures were similar to controls. By multivariate analysis, the primary negative determinants of CD were African American race and hyperparathyroidism; whereas, glomerular disease had a positive influence on capillary rarefaction \( R^2 = 64.2\% \) variance; \( P = 0.001 \).

Conclusion. Pediatric HD patients demonstrate a ‘structural deficit’ in CD but show preserved ‘functional integrity’. Capillary rarefaction, an early risk factor of incipient vascular calcification, was strongly associated with biomarkers of altered mineral metabolism. Further studies are warranted to determine the impact of optimizing blood pressure and metabolic control on changes in capillary rarefaction in young CKD patients.

Keywords: capillary rarefaction; microvascular disease; pediatric hemodialysis

Introduction

All-cause mortality in pediatric dialysis patients aged 0–19 years is at least 30 times that of the general pediatric population with cardiovascular disease (CVD) ranked as the leading cause of death in the pediatric dialysis population [1, 2]. Early markers of CVD risk include echocardiographic findings of abnormal cardiac geometry and large vessel anatomical changes related to hypertension and volume dysregulation. These are measured by increases in left ventricular mass, carotid intima-media thickness and large vessel stiffness (pulse wave velocity) [3–6].

Importantly, alterations in end-organ microvascular structure and function may preface the pathologic cascade into end-organ disease including diabetes, hypertension and kidney disease [7–9]. A recent focus on measuring tissue capillary rarefaction, defined as a decrease in the number of perfused capillaries in an area of tissue, has allowed an early assessment of microvascular function and tissue perfusion in various disease states including chronic kidney disease (CKD) [10–12]. Techniques developed to measure capillary rarefaction in the skin have been shown to accurately reflect central organ pathology including coronary artery disease and vascular calcification in dialysis patients [13–15]. Little is known of capillary rarefaction in hemodialysis (HD) patients and no studies have been published for pediatric patients [13, 16].

In this study, we sought to develop the technique of intravital nailfold capillaroscopy in pediatric HD patients and to assess its applicability as a non-invasive technique. The goal was to document capillary density (CD) in a pediatric dialysis population and its association with clinical and metabolic biomarkers of hypertension, cardiomyopathy and abnormal mineral metabolism.
Materials and methods

Study population and design

Nineteen pediatric patients with CKD stage 5 on chronic HD for >3 months and 20 healthy normotensive control subjects participated in this single-center, cross-sectional, 6-month longitudinal study. Since growth failure is prevalent in pediatric CKD patients, controls were closely matched for height-age, race and gender. The ‘height-age’, defined as the age equivalent to the 50th percentile for height and gender, is used to standardize physiologic and cardiovascular measurements in patients with growth failure. Of note, all subjects were either African American (AA) or Hispanic (H) which is consistent with the racial/ethnic demographic of the study site. In addition, control subjects had no significant prior or current medical illnesses or abnormal birth history such as prematurity or low birth weight. Patients were recruited from the pediatric dialysis unit and control subjects from the outpatient general pediatric clinics at Holtz Children’s Hospital/ Jackson Health System from April through December 2013. The study protocol was approved by the institutional review board of the University of Miami and informed consent was obtained from each subject. Patients were excluded if they had evidence of active vasculitis, congenital cardiac disease or were unable to sit quietly and allow the examination.

Clinical, demographic and biochemical data

Control subjects had sitting casual blood pressures measured at least twice in the clinic at the time of the nailfold capillaroscopy. Clinical history, demographic and body measurements were obtained at that encounter. Blood pressures were measured by an oscillometric method on the non-dominant and fistula-free arm (in HD patients) using a Dinamap® automated oscillometric device.

The primary renal disease in patients was classified as ‘glomerular’ when the diagnosis included predominant glomerular or vascular pathology such as nephrotic syndrome with focal glomerular sclerosis (FGS), systemic lupus erythematosus (SLE), IgA nephropathy or vasculitis. ‘Non-glomerular’ disease included those with congenital obstructive uropathy, renal dysplasia or reflux nephropathy.

In the HD patients, body measurements [height, weight and body mass index (BMI)] and blood pressures pre- and post-dialysis were obtained three times weekly. Laboratory assessments included at least monthly urea nitrogen, hemoglobin, ferritin, albumin, calcium, phosphorus and alkaline phosphatase. Multiple clinical and laboratory measurements were time-averaged over the 6-month study period. Intact parathyroid hormone (iPTH) and 25hydroxy vitamin D (25(OH)D) were assayed every 3 months during the study period. Biomarkers specific to cardiovascular risk including high-sensitivity C-reactive protein (hsCRP) and pro-brain natriuretic peptide (proBNP) were assayed once during the study period. Further, fibroblast growth factor 23 (FGF23), considered both a component of bone mineral metabolism and cardiac biomarker, was assayed using the C-terminal human FGF-23 ELISA (ImmunoLogic, San Clemente, CA). Medications, including the weekly dose of activated vitamin D and the number and class of antihypertensive medications, were recorded. Dialysis efficiency (Kt/V) was calculated as the ratio of the urea clearance (K) during time on dialysis (t) over the volume of distribution (V).

Nailfold capillary microscopy

CD measurements were performed by nailfold microscopy of the fourth finger on the non-dominant hand unless the patient had a vascular access (fistula or graft) in that arm. The technique was a modification of that described by Serné et al. [17]. The fourth finger was selected as it was less likely to have had prior trauma. Measurements were conducted in a quiet, temperature-controlled room (23.4 ± 0.4°C) after resting for ~15 min. Participants were fasting for 4–6 h and had abstained from caffeine-containing beverages for at least 24 h prior to the studies. HD patients took all their routine antihypertensive medications on the day of the exam, which was performed following the first or second dialysis session of the week.

Nailfold video capillaroscopy of the same visual field was performed in three phases: Phase 1 was a basal (un-stimulated) measure; Phase 2 was after inducing reactive hyperemia by applying a blood pressure cuff to the upper arm and inflating to 20 mmHg above the systolic BP for 2 min and then releasing it; Phase 3 was after the patients were allowed to rest for 15 min, the blood pressure cuff was re-applied and inflated to 60 mmHg for 2 min to achieve venous occlusion. Video recording of the microscopic examination for at least 1 min during each phase was captured with the Dino-Lite Premier AM7013MZT4® digital handheld microscope (Taiwan) at a magnification of ×400 using DinoCapture® 2.0 software. The technique was modified for pediatric patients after the method of Serné et al. [17]. Additional incident lighting was provided by an LED light source for patients with darker skin tones. The images were stored digitally and the capillaries were counted off-line by the same observer (A.E.R.).

For each phase, the number of erythrocyte-perfused capillaries was counted in three 0.6 square millimeter (mm²) field clips of the stored images using Dinocapture® 2.0 software. CD for each phase of the examination was the highest capillary count of the three fields assessed, expressed as number (n) of capillaries per mm². For verification of the counting methodology, two trained investigators counted the same fields in a blinded fashion from the preserved recordings. All readings were within 5% consistency between the two investigators (mean difference = 0.65 ± 2.3 capillaries/mm²; 95% confidence interval: −0.62 → 1.72).

Functional measures included capillary ‘recruitment’ and maximal ‘perfusion’ following stimulus maneuvers obtained during Phases 2 and 3. Percent (%) ‘recruitment’ was defined as the percent increase in perfused capillaries above basal CD during post-occlusive hyperemia (basal – post-hyperemia)/basal × 100. Percent (%) ‘perfusion’ was the post-hyperemia/maximal capillary density (MCD) post-venous occlusion × 100. The ‘structural’ component, in addition to absolute measures of CD, was assessed by the ‘% deficit in capillary density’ relative to controls. This was the difference in the average patient values compared with the control group expressed as a percent at each phase of stimulation and at baseline and 6 months [16].

Statistical analysis

All data sets were tested for normality with the D’Agostino and Pearson omnibus normality test. Continuous variables were expressed as the average ± SD when normally distributed or as the median and interquartile range for those that were not normally distributed. Intergroup comparisons were tested by one-way analysis of variance (ANOVA). Post-test comparisons for significance were performed by
the Kruskal–Wallis test for non-parametric data and by the Bonferroni method for parametric data as appropriate. Differences between two groups were analyzed with Fisher’s exact test. Univariate correlations were performed with Pearson’s correlation coefficient. Multivariate linear regression analysis was used to examine determinants of MCD based on P < 0.05 in univariate analysis and/or physiologic relevance. Graph Pad Prism version 6 for Windows (La Jolla, CA) and PAWS/SPSS® 18 (Chicago, IL) were the statistical programs used to perform the statistical analyses and to construct the graphs. A two-tailed P-value < 0.05 was considered significant.

Results

Demographic and clinical characteristics

Table 1 provides the demographic parameters of the control subjects and HD patients at initial entry into the study. Although the chronological age of the HD patients was significantly greater than that of controls, the ‘height-age’ was comparable between the two groups. The race/ethnicity of the study population included Hispanics (N = 18) and African Americans (N = 21) with similar distribution in each group (P = 0.11), which reflects the predominantly urban population served by our hospital. Among the patients, the primary disease classification was ‘glomerular’ in 14 (7 FSGS; 4 SLE; 2 vasculitis and 1 nephrotic syndrome). The remaining five patients had ‘non-glomerular’ disease including renal dysplasia (4) and reflux nephropathy (1). The majority of HD patients required at least two antihypertensive agents, usually including both an angiotensin antagonist and a calcium channel blocker. There was no association between MCD and antihypertensive medication use (R² = 0.005; P = 0.78). Except for the expected higher blood pressure measurements in the HD patients, all other parameters were comparable.

Laboratory and metabolic parameters

Table 2 shows the laboratory, cardiac and mineral biomarkers for the HD patients at study initiation and after 6 months. The patients demonstrated adequate measures of anemia control and dialysis efficiency (Kt/V). Levels of hsCRP and Pro-BNP were elevated above reference values.

| Parameter | Reference values | Initial N = 19 | 6 months N = 18 |
|-----------|-----------------|---------------|----------------|
| Hemoglobin (g/dL) | 12–14 | 11 ± 0.8 | 11 ± 0.6 |
| Kt/V ratio | >1.2 | 1.6 ± 0.2 | 1.6 ± 0.2 |
| hs-CRP (mg/L) | <1.0 | 0.9 (0.35, 2.65) | – |
| Pro-BNP (pg/mL) | 0–125 | 124 (539, 2270) | – |
| Total bicarbonate (mM/L) | 22–30 | 24 (22, 25) | 23 ± 2 |
| Albumin (g/dL) | 3.9–5.0 | 4.0 ± 0.4 | 3.9 ± 0.3 |
| Calcium (mg/dL) | 8.4–10.2 | 9.4 ± 0.6 | 9.3 ± 0.6 |
| Phosphorus (mg/dL) | 2.5–4.5 | 6.8 ± 2.0 | 6.6 ± 1.8 |
| Ca × P product (mg²/dL²) | <50 | 64 ± 20 | 62 ± 17 |
| 25(OH)D (ng/mL) | 30–100 | 31 ± 13 | 38 ± 19 |
| FGF-23 Log10 (RU/mL) | <2.4 | 3.96 (3.07, 4.78) | – |
| Intact PTH (pg/mL) | 15–65 | 483 ± 386 | 359 ± 202 |

The iPTH values were elevated at both time points, although within K/DOQI recommended ranges at 6 months [18]. The 25(OH)D levels were normal at both time points. As expected, FGF23 levels were markedly elevated in all HD patients.

Capillary microscopy

CD (capillaries/mm²) was significantly lower in HD patients compared with control subjects at each level of measurement including ‘basal’ (non-stimulated), post-occlusive hyperemia (recruitment) and post-venous occlusion (MCD) (Table 3 and Figure 1). Among the HD patients, only the MCD was significantly above basal levels at initial and 6-month measurements. Functional measurements including % recruitment and % perfusion were similar to control subjects (Table 3).

The capillary ‘deficit’ as shown in Table 3 and Figure 1 was significant in the HD patients compared with controls at each measure from basal to maximal stimulation. Moreover, the deficit in MCD increased substantially in the HD patients between both time points from 24 to 31%, a near-significant difference (P = 0.065).

Capillary rarefraction, mineral metabolism and cardiovascular risk markers

By univariate analysis, capillary rarefraction displayed an inverse association with both iPTH (r = −0.45; P = 0.005) and serum calcium (r = −0.39; P = 0.02) and a positive association with 25(OH)D levels (r = +0.36; P = 0.02). Neither serum phosphorus nor FGF23 associated with capillary rarefaction. Similarly, cardiac and inflammatory markers (hsCRP and Pro-BNP) did not associate with CD and were not analyzed further.

The only measure of systemic blood pressure in the study population that correlated significantly with capillary rarefaction was the mean arterial pressure (MAP) (r = 0.30; r = 0.02). However, in stepwise multivariate regression involving only the HD patients at baseline, MAP was not significantly associated with MCD and was not included in the final model. Similarly, interdialytic weight gain, as an indirect measure of volume status, did not reach significance when correlated to MCD (r = 0.32; P = 0.051).

Capillary rarefraction, demography and metabolic bone

Table 4 provides the linear and multivariate regression analyses comparing the demographic characteristics of the study population and their relative impact on the
Table 3. Capillary structure and function in pediatric HD patients

| Capillary function | % Recruitment | % Perfusion |
|--------------------|---------------|------------|
| HD patients—Initial | 13.3 ± 16     | 84.4 ± 14  |
| —6 months          | 10.1 ± 11     | 88.2 ± 11  |
| Controls           | 11.6 ± 15     | 84.2 ± 11  |
| P-values**         | 0.38          | 0.53       |

*Maximal versus basal and post-hyperemia.
**Controls versus HD patients.
were receiving supplements of vitamin D as well as an activated vitamin D (calcitriol) or one of its analogs (paricalcitol) following standard guidelines as part of their routine clinical management [18]. The levels of 25(OH)D correlated positively and significantly with the MCD, indicating improved capillary rarefaction with higher levels. Treatment with vitamin D may have exerted an attenuating effect on the capillaroscopic findings, because improving hyperparathyroidism may reduce the release of skeletal muscle minerals and reduce their participation in the process of vascular and coronary calcification [22].

Finally, despite exponentially elevated levels of the phosphaturic hormone FGF23 in our HD patients, we did not observe an association with capillary rarefaction. While cardiovascular morbidities including cardiac hypertrophy have been associated with elevations of FGF23 [23], this hormone does not appear to be directly involved in vascular calcification [24].

Microvascular rarefaction can be distinguished into both ‘structural’ and ‘functional’ components. ‘Functional’ rarefaction is defined as a decrease in the number of perfused vessels without reduction of the number of vessels anatomically present; whereas, ‘structural’ rarefaction refers to an actual reduction in the number of anatomically present vessels in the tissue [10]. Importantly, they can co-exist and ‘structural rarefaction’ may progress to ‘functional rarefaction’. It remains unclear at which point ‘structural rarefaction’ can be reversed and/or whether preservation of microvascular function may prevent end-organ damage. These are important potential targets for therapeutic interventions at all stages of CKD [11, 12].

In this study, the HD patients demonstrated a significant structural deficit in CD at each level of measurement including basal (non-stimulated), post-occlusion hyperemia and maximal stimulation with venous occlusion. The initial maximally stimulated deficit was 24%. After 6 months, it increased to 31%, although this did not reach significance. These findings are consistent with previous studies in patients with hypertension and normal renal function [25, 26] and CKD patients [16]. Importantly, the ‘functional measures’ of recruitment and perfusion were similar in patients compared with controls, suggesting a preservation of microvascular function. This could be due to the young age of the patients and/or the effect of the antihypertensive medications they were receiving including angiotensin antagonists believed to protect the microvasculature [27].

The traditional strong association of capillary rarefaction with systemic blood pressure was not apparent in our HD patients who were clearly hypertensive. This may be due to the small numbers of patients, but, may also highlight the minimal effect of blood pressure on capillary rarefaction in this group of CKD patients who were on longstanding antihypertensive medications. The role of endothelial dysfunction should also be addressed because post-occlusion hyperemia may be an indirect measure of endothelial function and was clearly lower in our patients compared with controls [28]. Further longitudinal studies with attention to parameters of vascular stiffness and endothelial function are required.

Of interest, in our cohort of dialysis patients, the trend was for those with primary glomerular disease to have less capillary rarefaction than those with obstructive uropathy and/or renal dysplasia. Although the classification of glomerular disease paradoxically included those with a primary diagnosis of vasculitis, they also, by virtue of having acquired disease, had shorter lifetime exposure to CKD. In contrast, those with congenital disease had had exposure since birth. Moreover, those with glomerular disease were primarily female while those with congenital disease were primarily male and African American. This suggests potential genetic and/or epigenetic influences on capillary rarefaction in children with CKD that may influence their progression to ESRD [11, 29].

This study has a number of limitations. First, although it appeared that the capillary deficit was increasing over time, our observation period was too short to determine any true difference. Second, the issue of AA race and female gender could not be clearly determined in this study. Our South Florida demographic is predominantly of Hispanic and African ethnicity and, consequently, our study subjects did not include any of non-Hispanic white race/ethnicity. Similarly, female gender was associated with increased CD as reported previously [17]. The difference in the measurements in the AA subjects does not appear to be a technical limitation because our results
are similar to other observations in which blacks were specifically excluded [30] or included [31].

In conclusion, this study demonstrates significant structural abnormalities in capillary rarefaction in young HD patients with some evidence for preserved microvascular function. The strong association with disordered mineral metabolism and poorly controlled hyperparathyroidism is ominous for impending microvascular calcification. Current screening practices focus on identifying cardiac and large vessel disease; whereas, early recognition and treatment of microvascular rarefaction may have more potential for improving long-term prognosis in these vulnerable patients. Hence, there is a need to standardize these techniques with a goal towards incorporating the measurement of capillary rarefaction into clinical practice.

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