Skin-Autofluorescence, a Measure of Tissue Advanced Glycation End-Products (AGEs), is Related to Diastolic Function in Dialysis Patients

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ABSTRACT

Background: Diastolic dysfunction is a frequent cause of heart failure, particularly in dialysis patients. Advanced glycation endproducts (AGEs) are increased in dialysis patients and are suggested to play a role in the development of diastolic dysfunction. The aim of our study was to assess whether AGE accumulation in dialysis patients is related to the presence of diastolic dysfunction.

Methods and Results: Data were analyzed from 43 dialysis patients, age 58 ± 15 years, of whom 65% were male. Diastolic function was assessed using tissue velocity imaging (TVI) on echocardiography. Tissue AGE accumulation was measured using a validated skin-autofluorescence (skin-AF) reader. Plasma N^{ϵ}-(carboxymethyl)lysine (CML) and N^{ϵ}-(carboxyethyl)lysine (CEL) were measured by stable-isotope-dilution tandem mass spectrometry. Plasma pentosidine was measured by high-performance liquid chromatography. Skin-AF correlated with mean E′ ($r = -0.51, P < .001$), E/A ratio ($r = -0.39, P = .014$), and E/E′ ($r = 0.38, P = .019$). Plasma AGEs were not significantly associated with diastolic function. Multivariable linear regression analysis revealed that 54% of the variance of average E′ was explained by age ($P = .007$), dialysis type ($P = 0.016$), and skin-AF ($P = .013$).

Conclusions: Tissue AGEs measured as skin-AF, but not plasma AGE levels, were related to diastolic function in dialysis patients. Although this may support the concept that tissue AGEs explain part of the increased prevalence of diastolic dysfunction in these patients, the ambiguous relation between plasma and tissue AGEs needs further exploring. (*J Cardiac Fail 2008;14:596–602*)

Key Words: Heart failure, tissue velocity imaging, carboxymethyllysine.

Systolic dysfunction is commonly recognized as the main cause of heart failure. However, approximately 50% of the patients with chronic heart failure have a preserved systolic function.[1] In most of these patients, heart failure is caused by diastolic dysfunction.[2] Recently, it became evident that the prognosis of patients with diastolic heart failure is nearly as poor as the prognosis of patients with systolic heart failure.[2–4] Despite the magnitude of this problem, little is known about the pathophysiologic background of diastolic dysfunction.

Several mechanisms underlying diastolic dysfunction have been proposed.[5–7] Diastolic dysfunction is generally associated with increased myocardial stiffness, which may be caused by modifications of collagen in the extracellular matrix. One important modification of collagen is increased cross-linking by the formation of advanced glycation endproducts (AGEs). These are carbohydrate and lipid dependent modifications of protein formed by oxidative or nonoxidative reactions.[8]

AGE accumulation occurs during life, but an increased level of AGEs has been found in patients with diabetes and renal failure. Particularly, dialysis patients are known for increased levels of AGEs, which were also

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independently associated with an impaired survival.[9] Interestingly, a frequent echocardiographic finding in dialysis patients is diastolic dysfunction. The prevalence of diastolic dysfunction in dialysis patients varies from 25% to 87% depending on definitions used and the patients included.[10,11] Diastolic dysfunction predisposes to the development of heart failure and is strongly related with outcome in dialysis patients.[12] We hypothesized that increased AGE accumulation in dialysis patients explains part of the increased prevalence of diastolic dysfunction; therefore, we analyzed the relation between tissue and plasma AGEs and diastolic function in dialysis patients.

Methods

Patients and Study Design

In this cross-sectional study, all patients receiving dialysis treatment at the Dialysis Center Groningen ≥18 years old were eligible to participate. Both hemodialysis and peritoneal dialysis patients were included. Exclusion criteria were a myocardial infarction within the last month, significant valvular disease, pacemaker use, sustained or accepted atrial fibrillation, active endocarditis, active myocarditis, active pericarditis, acute heart failure, heart transplant, and secondary (nonidiopathic) cardiomyopathies. Non-Caucasian patients were excluded from analysis because the autofluorescence reader was validated only in Caucasians. Study visits as well as blood collections were performed before hemodialysis therapy. In case of peritoneal dialysis, patients' blood was withdrawn at their study visit. Using standard laboratory techniques blood was analyzed for hemoglobin (Hb), HbA1c, total protein, albumin, calcium, phosphate, triglycerides, total cholesterol, and low-density lipoprotein (LDL). Furthermore, we analyzed the mean value of Kt/V per week (marker for dialysis quality based on urea clearance), which was expressed as a percentage of the Kt/V recommended by the Kidney Disease Outcomes Quality Initiative (K-DOQI) adequacy guidelines for dialysis therapy (Kt/V >3.6 for hemodialysis patients; Kt/V >2.0 for peritoneal dialysis patients). Clinical measurements were all performed on the same day as echocardiography, and included blood pressure and heart rate. In hemodialysis patients, blood pressure obtained before dialysis therapy was used for analysis. Current use of medication was extracted from medical records. History of cardiovascular disease (CVD) and family history of CVD were based on a documented or reported history of myocardial infarction, angina pectoris, cerebrovascular accident, transient ischemic attack, pulmonary embolus, venous thrombosis, or intermittent claudication in the medical history of the patient or first- and second-degree relatives, respectively. This study protocol complies with the Declaration of Helsinki, and was approved by the institutional review committee of the University Medical Center Groningen. All patients signed written informed consent.

Skin-Autofluorescence

Tissue AGE accumulation was assessed using a validated skin-autofluorescence (skin-AF) reader (AGE-Reader; patent PCT/NL99/00607; DiagnOptics BV, Groningen, The Netherlands) described previously.[9,13] In short, the AGE reader illuminates a skin surface of approximately 4 cm^2, guarded against surrounding light, with an excitation light source between 300 and 420 nm (peak excitation ~370 nm). Light from the skin is measured with a spectrometer in the 300- to 600-nm range, using 200-μm glass fiber. As a measure of skin-AF, the ratio between emission and excitation was calculated in arbitrary units (a.u.) by dividing the area under the curve between 420 and 600 nm by the area under the curve between 300 and 420 nm, and multiplying by 100. Skin-AF was measured at the volar side of the lower arm at approximately 10 to 15 cm below the elbow fold. Care was taken to perform the measurement at normal skin site (ie, without visible vessels, scars, lichenification, or other skin abnormalities). Intraobserver variation of repeated AFR measurements on 1 day was 6%.

Plasma N^{ε}-(carboxymethyl)lysine and N^{ε}-(carboxyethyl)lysine by LC-MS/MS

Plasma N^{ε}-(carboxymethyl)lysine (CML) and N^{ε}-(carboxyethyl)lysine (CEL) were determined by stable-isotope dilution tandem mass spectrometry (LC-MS/MS) as described previously.[14] In short, CML and CEL were liberated from plasma proteins by acid hydrolysis after addition of deuterated CML and CEL as internal standards. Chromatographic separation was performed by gradient-elution reversed-phase chromatography with a mobile phase containing 5 μmol/L nonafluoropentanoic acid as ion-pairing agent. Mass transitions of 205.1 > 384.1 and 219.1 > 384.1 for CML and CEL, respectively, and 209.1 > 388.1 and 223.1 > 388.1 for their respective internal standards were monitored in positive-ion mode. CML and CEL were separated by baseline resolution with a total analysis time of 21 minutes. Within-day and between-day coefficients of variation were <4.4% and <3.2% for CML, and <6.8% and <7.3% for CEL.

Pentosidine by High-Performance Liquid Chromatography

Pentosidine levels were measured by high-performance liquid chromatography as described previously by Izuhara et al.[15] Briefly, a 50-μL solution of acid hydrolysate of plasma was injected into an high-performance liquid chromatography system and separated on a C18 reverse-phase column (Waters, Tokyo, Japan). The effluent was monitored using a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan) at an excitation-emission wavelength of 335/385 nm. Synthetic pentosidine was used to obtain a standard curve. The limit of detection was 5 pmol of pentosidine per milliliter of plasma. Normal values in 4 healthy subjects averaged 0.114 ± 0.011 μmol/L, with a coefficient of variation of 5.48% ± 0.81% on 4 different days.

Echocardiography

Patients underwent 2-dimensional echocardiography, including color flow mapping 2-dimensional−guided M-mode, blood pool, and tissue Doppler echocardiography. Echocardiography was performed by experienced cardiac technicians using a General Electric VIVID 7 system with a 2.5-mHz probe. Measurements included left ventricular and atrial dimensions, the peak early (E) and late (A) diastolic filling velocities, isovolumetric relaxation time, deceleration time (slope) of the early peak filling. Furthermore, using tissue velocity imaging (TVI), early diastolic velocity (E′) was measured on the lateral, septal, anterior, and inferior wall areas, and subsequently averaged. E/E′ was calculated by dividing the peak early diastolic filling (E) by the average E′ measured using TVI. Systolic dysfunction was defined as an estimated left ventricular ejection fraction (LVEF) ≤45%. Diastolic dysfunction was defined as an E′ <8 cm/s. E′ values could not