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Cardiovascular autonomic neuropathy associates with nephropathy lesions in American Indians with type 2 diabetes

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ABSTRACT

Aims: Cardiovascular autonomic neuropathy (CAN) predicts clinical diabetic nephropathy (DN). We investigated the relationship between DN structural lesions and CAN.
Methods: Sixty three Pima Indians with type 2 diabetes underwent kidney biopsies following a 6-year clinical trial testing the renoprotective efficacy of losartan *vs.* placebo. CAN was assessed a median 9.2 years later. CAN variables included expiration/inspiration ratio (E/I), standard deviation of the normal R-R interval (sdNN), and low and high frequency signal power and their ratio (LF, HF, LF/HF); lower values reflect more severe neuropathy. Associations of CAN with renal structural variables were assessed by linear regression adjusted for age, sex, diabetes duration, blood pressure, HbA1c, glomerular filtration rate, and treatment assignment during the trial.
Results: Global glomerular sclerosis was negatively associated with sdNN (partial $r = -0.35$, $p = 0.01$) and LF ($r = -0.32$, $p = 0.02$); glomerular basement membrane width was negatively associated with all measures of CAN except for LF/HF ($r = -0.28$ to -0.42, $p < 0.05$); filtration surface density was positively associated with sdNN, LF, and HF ($r = 0.31$ to 0.38, $p < 0.05$); and cortical interstitial fractional volume was negatively associated with HF ($r = -0.27$, $p = 0.04$).
Conclusions: CAN associates with DN lesions.

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1. Introduction

Cardiovascular autonomic neuropathy (CAN) and diabetic nephropathy (DN) are common complications of diabetes with major impacts on morbidity and mortality (Pop-Busui et al., 2010). CAN is the consequence of damage to and loss of the small, unmyelinated nerve fibers that innervate the heart and blood vessels, resulting in abnormalities of heart rate control and vascular dynamics; the pathophysiology is similar to that of peripheral neuropathy. Presence of CAN, which may be documented by abnormal cardiovascular reflex tests and reduction in heart rate variability (HRV), increases the risk of all-cause mortality 3-fold in those with diabetes. DN is the leading cause of end-stage renal disease worldwide (Maser, Mitchell, Vinik, & Freeman, 2003; Tuttle et al., 2014), and its interrelationship with CAN remains unclear. Persons with either type 1 or type 2 diabetes who also have CAN have a faster rate of renal function decline than those who do not (Orlov et al., 2015; Sundkvist & Lilja, 1993; Tahrani et al., 2014; Yun et al., 2015), suggesting an association between these complications (Maguire et al., 2007; Molgaard, Christensen, Sorensen, Christensen, & Mogensen, 1992; Moran et al., 2004; Orlov et al., 2015; Spallone et al., 1994; Sundkvist & Lilja, 1993; Tahrani et al., 2014; Weinrauch, Kennedy, Gleason, Keough, & D'Elia, 1998; Yun et al., 2015; Zander et al., 1989) which may be attributable to CAN-induced changes in renal hemodynamics (Molgaard et al., 1992; Orlov et al., 2015; Spallone et al., 1994; Weinrauch et al., 1998). The structural underpinnings of this relationship have not been assessed.

In the present study, we examined the association between CAN and preceding DN lesions in Pima Indians with type 2 diabetes using standard measures of CAN and morphometry of kidney tissue obtained via protocol research biopsy.

Conflicts of interest: The authors have nothing to disclose.

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2. Subjects

From 1965–2007, Pima Indians from the Gila River Indian Community participated in a longitudinal study of diabetes and its complications. We selected 169 adults with type 2 diabetes from this population to participate in a randomized clinical trial testing the renoprotective efficacy of losartan in early DN (ClinicalTrials.gov number, NCT00340678). Ninety-one subjects had normal urinary albumin excretion (albumin/creatinine ratio [ACR] <30 mg/g) at baseline and 78 had microalbuminuria (ACR = 30–299 mg/g). Glomerular filtration rate (GFR) was measured annually throughout the trial, and the pre-specified primary endpoint was a decline in GFR to ≤60 ml/min or to half the baseline value in subjects who entered the study with a GFR <120 ml/min. At the end of the six-year clinical trial, 111 of the subjects underwent kidney biopsy (60 with normoalbuminuria and 51 with microalbuminuria at baseline) to determine whether treatment was associated with preservation of kidney structure (Weil et al., 2013). Among subjects with microalbuminuria at baseline, those who received losartan had lower mesangial fractional volume on average than those who received placebo, suggesting that losartan preserved some aspects of normal glomerular structure in early DN, although losartan did not significantly affect the primary outcome, i.e., decline in GFR.

We assessed CAN under a separate protocol a median of 9.3 years (range = 6.9–12.3 years) after kidney biopsy by cardiovascular reflex testing and heart rate variability studies in 63 participants. Subjects who died (n = 11), developed end-stage renal disease (n = 15), were lost to follow-up (n = 20) or did not have full clinical data available at both the biopsy and the CAN evaluation (n = 2) were excluded from the analysis.

Clinical data were collected within a median of 46 days (range = 7–216 days) of the kidney biopsy and again at the time of the CAN examination. At the exam nearest the kidney biopsy, 17 (49%) of the participants in the normoalbuminuria group and 16 (57%) in the microalbuminuria group were receiving losartan as part of the clinical trial. Upon completion of the clinical trial, participants received all medical care at the direction of their primary physicians.

This study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases. Each subject signed an informed consent document.

3. Materials and methods

3.1. Clinical and anthropometric measures

BMI was defined as weight divided by the square of height (kg/m^2). Blood pressure was measured while the subject was resting in the seated position; mean arterial pressure (MAP) was calculated as (2*diastolic blood pressure + systolic blood pressure)/3. HbA1c was measured by HPLC (Tosoh, Tokyo, Japan). HPLC was also used to measure urinary clearance of non-radioactive iothalamate for GFR determination (Waters, Milford, Massachusetts) (Myers et al., 1995). Urinary albumin concentration was measured by nephelometric immunoassay and urinary creatinine by a modified Jaffé reaction (Siemens, Erlangen, Germany) (Chasson, Grady, & Stanley, 1960; Vasquez et al., 1984). Urinary albumin concentration below the detection limit of the assay (≤6.8 mg/L) was set to 6.8 mg/L in the analyses. Inflammatory factors such as C-reactive protein, interleukin-6, and TNF-α were not measured in study participants.

3.2. Morphometric measures of DN

We estimated renal structural parameters using quantitative morphometric methods on digital images obtained by masked unbiased random sampling of light and electron microscopy sections (Weibel, 1979; Weil et al., 2013). Parameters included in this study were mean glomerular volume, glomerular basement membrane (GBM) width, mesangial fractional volume, glomerular filtration surface density, total filtration surface per glomerulus, cortical interstitial fractional volume, percent globally sclerotic glomeruli, number of podocytes per glomerulus, podocyte foot process width, percent podocyte detachment, and percentage of normally fenestrated endothelium (Fioretto, Steffes, & Mauer, 1994; Mauer et al., 1984; Pagtalunan et al., 1997; Weil et al., 2012, 2013). An equation that takes into account the smaller diameter of sclerotic glomeruli, and the consequent difference in the probability of encountering a sclerotic or non-sclerotic glomerulus in a random cross-section, was used to calculate the percentage of sclerotic glomeruli (Tan et al., 2010). Glomerular variables were calculated for each individual as the mean of all glomeruli evaluated (3 ± 1) for that individual.

3.3. CAN measures

We performed standardized CAN evaluations (deep breathing test and heart rate variability) on all subjects after an overnight fast as described previously (Jaiswal et al., 2014). Subjects were asked to avoid vigorous exercise for 24 h and caffeine and tobacco products for 8 h prior to testing, and to hold any medicines on the day of testing until the evaluation was completed. The median fasting plasma glucose concentration at time of testing was 191 mg/dL (range = 94–422 mg/dL). None of the subjects experienced a hypoglycemic episode within 24 h prior to testing. Electrocardiogram (ECG) recordings were obtained after a 20-min rest in the supine position using a physiologic monitor (Nightingale PPM2, Zoe Medical Inc., Topsfield, MA). Data were collected during a resting study (5 min) and a standard deep breathing test paced at 6 breaths/min (5 s of inspiration and 5 s of expiration), which is one of the most sensitive cardiovascular reflex tests for CAN assessment (Jaiswal et al., 2014). We analyzed heart rate variability studies according to current guidelines using the continuous wavelet transform methods with the ANX 3.1 (ANSAR Inc., Philadelphia, PA) (Bernardi et al., 2011). This method incorporates respiratory activity in the formula and is considered superior for the analysis of non-stationary signals compared with the Fourier transform as it performs a time-frequency decomposition of the signal (Pichot et al., 1999).

The following measures of CAN were predefined and analyzed as outcomes of interest: a) the expiration/inspiration (E/I) ratio, calculated as the longest R-R interval (in milliseconds) during expiration divided by the shortest R-R interval during inspiration, averaged for the six respiration cycles, and b) time- and frequency-domain measures of heart rate variability (HRV) using recordings during rest. These HRV measures included the standard deviation of the normal R-R interval (sdNN), low frequency signal power (LF, 0.04–0.15 Hz), high frequency signal power (HF, 0.15–0.40 Hz), and the LF/HF ratio.

The E/I ratio assesses the magnitude of sinus arrhythmia, a physiologic response mediated predominantly by the parasympathetic nervous system (Bernardi et al., 2011). The sdNN, a measure of overall heart rate variability, and the HF are largely mediated by parasympathetic activity. LF is affected by both sympathetic and parasympathetic modulation (Reyes del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013). Generally, increased sympathetic outflow leads to a reduction of both LF and HF. It is important to note that LF and HF are not necessarily indicative of autonomic activity, but rather autonomic regulation.

There are currently no established, validated cut-points on the measures of HRV to define the presence of CAN, but lower values reflect more advanced autonomic impairment. Therefore, we used continuous measures of CAN in the analyses.

3.4. Statistical analysis

Data are presented as mean ± standard deviation or median (IQR). Since the clinical variables were recorded at the kidney biopsy