Association between cardiovascular autonomic neuropathy and left ventricular hypertrophy in diabetic haemodialysis patients

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Abstract

Background. Patients with diabetic nephropathy are likely to have neurological complications including cardiovascular autonomic dysfunction, which is related to increased risk of mortality. We investigated whether cardiovascular autonomic neuropathy is associated with left ventricular hypertrophy (LVH) in diabetic haemodialysis patients.

Methods. Holter electrocardiography was carried out for 24 h with time and frequency domain analyses of heart rate variability in 154 diabetic (age 62±11 years) and 63 non-diabetic haemodialysis patients (62±10 years). The left ventricular mass index (LVMI) was determined by echocardiography. We used the percentage of differences exceeding 50 ms between adjacent normal RR intervals (pNN50) in time domain analysis and the power in the high-frequency range (HF: 0.15–0.40 Hz) in frequency domain analysis as indicators of parasympathetic activity.

Results. The mean LVMI was greater in diabetic than in non-diabetic patients (168±63 vs 144±54 g/m², P<0.01). LVMI inversely correlated with pNN50 (r=−0.270, P=0.0007, n=154) and HF (r=−0.277, P=0.0005, n=154) in diabetic patients, but not in non-diabetic patients. By multiple logistic analysis, LVH was strongly associated with pNN50 (odds ratio 0.088; 0, <2%; 1, ≥2%) and HF (odds ratio 0.058; 0, <500 ms²; 1, ≥500 ms²) in diabetic patients.

Conclusions. Impaired parasympathetic activity, which indicates cardiovascular autonomic neuropathy, was associated with the presence of LVH in diabetic haemodialysis patients. The co-existence of cardiovascular autonomic neuropathy and LVH may be one of the key factors for the high incidence of cardiovascular events in diabetic haemodialysis patients.

Keywords: diabetes mellitus; haemodialysis; heart rate variability; left ventricular hypertrophy; parasympathetic activity

Introduction

Left ventricular hypertrophy (LVH) is relatively common in end-stage renal disease (ESRD), with a prevalence of nearly 75%. Cross-sectional studies have shown LVH to be an independent risk factor for shortened survival in ESRD patients [1]. Diabetic nephropathy is the leading cause of ESRD requiring renal replacement therapy in Japan. Patients with diabetic nephropathy are likely to have neurological complications of diabetes including cardiovascular autonomic dysfunction [2]. Defined as impairment of the cardiac parasympathetic and sympathetic nervous system, cardiovascular autonomic dysfunction is associated with increased risk of mortality, particularly cardiac deaths, in diabetic patients [3–5]. It would influence the prognosis of diabetic ESRD patients to have cardiovascular autonomic dysfunction as well as LVH as their complications. However, we know of no previous study examining the relationship of cardiovascular autonomic neuropathy to LVH in diabetic haemodialysis patients. Time and frequency domain analyses of heart rate variability have been used extensively for non-invasive assessment of the autonomic nervous control of cardiovascular function. In time domain measures, the percentage of differences between adjacent normal RR intervals exceeding 50 ms over the entire 24 h electrocardiographic (ECG) recording (pNN50) is one of the representative parameters...
showing cardiac parasympathetic activity [6]. The efferent vagal activity is a major contributor to the power in the high-frequency range (HF: 0.15–0.40 Hz) in frequency domain measures [7,8], while the power in the low-frequency range (LF: 0.04–0.15 Hz) is thought to be a parameter that includes both sympathetic and parasympathetic influences [9,10]. Our present findings showed an association between impaired parasympathetic activity and LVH in diabetic haemodialysis patients by using time and frequency domain analyses of heart rate variability.

**Subjects and methods**

**Subjects and study protocol**

In this study, we enrolled 217 patients who had undergone maintenance haemodialysis for >6 months at Toujinkai Hospital. Patients were excluded if they had (i) a history of angina pectoris, myocardial infarction or moderate to severe congestive heart failure (New York Heart Association grades III–IV); (ii) arrhythmias such as atrial fibrillation, atrial flutter, or premature atrial or ventricular ectopic beats exceeding 100 per day; or (iii) non-compliance with fluid intake restrictions (body weight gain between dialyses exceeding 5% of ‘dry weight’). Of 217 patients, 154 had diabetes mellitus; the other 63 had no present or previous history of diabetes (Table 1). Mean duration of a diabetic history was 20.0±4.8 years. Diabetic nephropathy had been proven to be the cause of ESRD by renal biopsy examination in 126 of 154 diabetic patients (81.8%). In the other 28 patients, diabetes mellitus had been diagnosed before the occurrence of renal insufficiency, but the exact cause of ESRD had not been ascertained by renal biopsy examination.

Orthostatic hypotension, a clinical hallmark of cardiovascular autonomic neuropathy, was found in 33 of 154 diabetic patients (21.4%). The Ethics Committee for Human Research of Toujinkai Hospital approved this study, and all patients provided informed consent for participation.

Haemodialysis was performed three times weekly using a dialysate containing Na⁺ (140 mEq/l), K⁺ (2.0 mEq/l), Cl⁻ (110 mEq/l), Ca²⁺ (3.0 mEq/l), Mg²⁺ (1.0 mEq/l), HCO₃⁻ (30 mEq/l) and CH₃COO⁻ (10–15 mEq/l). Membranes used in the various dialysers included either cellulose triacetate (20% of dialyses in non-diabetic and 20% in diabetic patients; FB-190F, NIPRO, Tokyo), surface-modified regenerated cellulose (19% in non-diabetic and 18% in diabetic patients; AMBC-20X, Asahi Medical, Tokyo), polymethyl methacrylate (22% in non-diabetic and 23% in diabetic patients; FB-2.1F, Toray Medical, Tokyo) or polysulfone (39% in non-diabetic and 39% in diabetic patients; PS-1.9UW, Kawasaki Laboratory, Tokyo). Dialysis filter surface areas ranged from 1.8 to 2.1 m². Blood pressure was measured hourly during dialysis using a mercury sphygmomanometer. Mean blood pressure and pulse pressure were determined as the means of measurements obtained in eight different midweek haemodialysis sessions in which patients showed essentially the same increase in body weight, including the week in which Holter ECG was performed.

**Determination of left ventricular mass index**

A two-dimensionally guided M-mode echocardiogram was obtained for each patient using a single ultrasonic recorder (UF-8800, Fukuda Denshi, Tokyo) with a 3.5 MHz transducer on the day between midweek dialysis sessions within 2 weeks after the 24 h Holter ambulatory ECG monitoring. Measurements for M-mode-guided calculation of left ventricular mass [LVM; left ventricular internal end-diastolic and end-systolic dimensions (LVIdD and LVIds), interventricular septal wall thickness (IVST) and left ventricular posterior wall thickness (PWT)] were obtained according to the guidelines of the American Society of Echocardiography [11]. Relative left ventricular wall thickness (rLWT) was calculated as 2 × PWT/LVIdD. LVM was calculated according to a formula described by Devereux et al. [12]:

\[ LVM = 1.04 \times (LVIdD + IVST + PWT)^3 - LVIdD^3 \] – 13.6 g.

LVM was normalized to body surface area as the LVM index (LVTI). Criteria for LVH were an LVTI exceeding 34 g/m² in men or 110 g/m² in women [11]. The mean coefficient of variation (CV) of LVM measurement was 2.14±0.90% (n = 10).

**Analysis of heart rate variability**

All patients underwent Holter ambulatory ECG monitoring (FM-100, Fukuda Denshi) for 24 h from the day before the midweek dialysis session to 1 h before the start of dialysis, within 2 weeks before cardiac echocardiography. Holter

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**Table 1. Characteristics of diabetic and non-diabetic patients**

<table>
<thead>
<tr>
<th></th>
<th>Diabetic (n=154)</th>
<th>Non-diabetic (n=63)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male, female)</td>
<td>88, 66</td>
<td>33, 30</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62±11</td>
<td>62±10</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of dialysis (months)</td>
<td>72±58</td>
<td>116±92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MBP before dialysis (mmHg)</td>
<td>99.1±10.7</td>
<td>96.9±11.7</td>
<td>NS</td>
</tr>
<tr>
<td>MBP after dialysis (mmHg)</td>
<td>87.0±9.4</td>
<td>86.3±12.3</td>
<td>NS</td>
</tr>
<tr>
<td>PP before dialysis (mmHg)</td>
<td>71.7±13.2</td>
<td>62.6±11.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PP after dialysis (mmHg)</td>
<td>69.1±14.7</td>
<td>64.3±12.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cardiothoracic ratio (%)</td>
<td>51.3±4.9</td>
<td>51.5±5.7</td>
<td>NS</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.319±0.034</td>
<td>0.323±0.035</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>4.0±0.4</td>
<td>4.0±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Serum haemoglobin (g/l)</td>
<td>105±10</td>
<td>106±9</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma BNP (pg/ml)</td>
<td>362±305</td>
<td>389±360</td>
<td>NS</td>
</tr>
<tr>
<td>LVIdD (mm)</td>
<td>48.8±6.9</td>
<td>48.7±7.4</td>
<td>NS</td>
</tr>
<tr>
<td>LVIds (mm)</td>
<td>30.8±7.5</td>
<td>29.7±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>LVFS (%)</td>
<td>37.3±9.8</td>
<td>39.1±9.2</td>
<td>NS</td>
</tr>
<tr>
<td>IVST (mm)</td>
<td>12.0±3.4</td>
<td>10.9±3.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PWT (mm)</td>
<td>9.4±2.3</td>
<td>8.5±2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rLVWT</td>
<td>0.40±0.14</td>
<td>0.36±0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>168.3±63.2</td>
<td>144.5±53.8</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

MBP = mean blood pressure; PP = pulse pressure; BNP = B-type natriuretic peptide; LVIdD = left ventricular internal end-diastolic dimension; LVIds = left ventricular internal end-systolic dimension; LVFS = left ventricular fractional shortening; IVST = interventricular septal wall thickness; PWT = left ventricular posterior wall thickness; rLVWT = relative left ventricular wall thickness; LVMI = left ventricular mass index.
recording were scanned with Holter processing equipment (SCM-6000, Fukuda Denshi), and QRS complexes were identified and labelled. Heart rate variability parameters were analysed by use of commercially available software [HPS-RRLOP (1), Fukuda Denshi]. Special indices of heart rate variability were computed by fast Fourier transformation from 512 consecutive normal RR intervals of the recording, with application of a Hanning window to minimize spatial leakage. Power spectra from sequential pre-specified segments were averaged every hour and for the entire 24 h time period. The following frequency domain measures were assessed: (i) total power (0.0–1.0 Hz); (ii) power in the low-frequency component or LF (0.04–0.15 Hz); (iii) power in the high-frequency component or HF (0.15–0.40 Hz); and (iv) the LF/HF ratio. Measurement of total power, LF and HF was carried out in absolute values of power (ms²). As time domain measures, we used the number of total normal RR intervals during the 24 h monitoring period (total NN), the SD of the average normal RR interval for all 5 min segments of a 24 h ECG recording (SDANN) and the percentage of differences between adjacent normal RR intervals exceeding 50 ms over the entire 24 h ECG recording (pNN50). Analysis of heart rate variability was performed by highly experienced employees of Fukuda Denshi kept unaware of details of this study. Mean values of CV in measures of heart rate variability were 3.29±0.88% in LF (n=10), 3.54±0.84% in HF (n=10), 3.36±0.93% in SDANN (n=10) and 2.37±0.84% in pNN50 measurements (n=10), respectively.

Biochemical measurements

Blood samples (5 ml) were obtained just before initiation of haemodialysis in the same week as the echocardiographic examination. Plasma B-type natriuretic peptide (BNP) concentration was measured with a sensitive immunoradiometric assay (Shionoria BNP, Shionogi, Osaka). Intra- and inter-assay variations of the assay were 5.3 and 5.9%, respectively. Haematocrit and serum concentrations of haemoglobin and albumin were determined as the mean values of four different measurements within a 2 month period, which included the day of echocardiographic examination.

Ambulatory blood pressure monitoring

Diurnal 24 h ambulatory blood pressures were recorded (FM-200, Fukuda Densi) between midweek dialysis sessions within 30 days after 24 h Holter ambulatory ECG monitoring, and the data were analysed (SCM-6000) in diabetic or non-diabetic patients with or without LVH. The presence of LVH was determined according to the criteria of echocardiographic examination. Difference in heart rate variability between diabetic and non-diabetic patients

Time domain analysis of heart rate variability showed that mean pNN50 and SDANN were lower in diabetic than in non-diabetic patients, although the total number of NN intervals during 24 h did not differ between the two groups (Table 2). In frequency domain analysis of heart rate variability, mean values of total power, LF and HF were reduced in diabetic patients compared with non-diabetic patients, although the LF/HF ratio did not differ between the two groups. Figure 1 shows typical patterns of power spectra of heart rate variability between diabetic and non-diabetic patients.

Statistical analysis

All values are expressed as mean±SD. Differences between groups were evaluated by one-way analysis of variance, followed by application of Duncan’s new multiple range test. Relationships between LVH and clinical parameters in diabetic or non-diabetic patients first were evaluated by univariate logistic analysis; then the association of LVH with other continuous or categorical data collected in patients with or without diabetes mellitus was analysed using a multiple logistic model with a dichotomous response variable for presence/absence of LVH according to LVMI criteria. Selection of covariates included in the final model was carried out using backward elimination, forward selection and stepwise selection. As a measure of relative risks for LVH, odds ratios and their 95% confidence intervals (CIs) were presented in order to summarize the effects of each covariate. All statistical tests were two-sided, with significance accepted at a z level of 0.05.
variability during 24 h in diabetic and non-diabetic patients.

Heart rate variability and LVH

In diabetic patients, LVMI correlated negatively with pNN50 or HF (Figure 2), and positively with LF/HF (r = 0.263, P = 0.001, n = 154), but did not correlate with mean blood pressure, pulse pressure or plasma BNP concentration. In contrast, in non-diabetic patients, LVMI did not correlate with any parameters of heart rate variability in non-diabetic patients.

The results of univariate logistic analysis (Table 3) indicated that LVH in diabetic patients was significantly associated with parameters of heart rate variability such as pNN50, HF and LF/HF, but not with blood pressure, pulse pressure or plasma BNP concentration. In non-diabetic patients, however, LVH tended to be related to mean blood pressure after dialysis, pulse pressure after dialysis, and plasma BNP concentration, but not to parameters of heart rate variability.

In multiple logistic analysis (Table 4), odds ratios of pNN50 and HF to LVH in diabetic patients were 0.787 and 0.993, respectively when values of pNN50 or HF were treated as continuous variables. The odds ratio of <2% pNN50 vs ≥2% pNN50 to LVH was 11.364 (3.690–34.433), and the odds ratio of <500 ms² vs ≥500 ms² to LVH was 17.24 (5.495–55.556) in diabetic patients. LF/HF ratio was not chosen as one of the covariates in multiple logistic models in the diabetic group by the selection procedures. In non-diabetic patients, the odds ratio of plasma BNP concentration to LVH was 1.002, and no significant association was found between LVH and parameters of heart rate variability.

![Fig. 1.](image-url) Typical examples of power spectra of heart rate variability during 24 h. (A) A 56-year-old non-diabetic man: total power (0.04–0.15 Hz), 6364 ms²; LF (0.04–0.15 Hz), 1535 ms²; HF (0.15–0.40 Hz), 665 ms². (B) A 64-year-old diabetic woman: total power, 880 ms²; LF, 31 ms²; HF, 21 ms².
Ambulatory blood pressure in diabetic patients with or without LVH and impaired parasympathetic activity

Mean daytime and night time systolic blood pressures did not differ between diabetic patients with or without LVH (daytime, 140 ± 12 mmHg, n = 30 vs 142 ± 11 mmHg, n = 25; night time, 151 ± 9 mmHg, n = 30 vs 153 ± 9 mmHg, n = 25). Mean diastolic blood pressure also did not differ between these two subgroups during

Discussion

In the present study, diabetic haemodialysis patients showed lower mean values than non-diabetic haemodialysis patients in pNN50 and SDANN in time.
domain analysis of heart rate variability, and in total power, LF and HF in frequency domain analysis. The decrease in heart rate variability in diabetic patients appeared to be derived mainly from reduction of parasympathetic activity, since pNN50 and HF are parameters indicative of such activity and parasympathetic activity is significantly related to components of SDANN, total power and LF [6]. Further, logistic regression analysis showed that reduced parasympathetic activity was related strongly to the presence of LVH in diabetic haemodialysis patients, but not in non-diabetic patients. These results indicate that impaired parasympathetic function, most probably induced by diabetic autonomic neuropathy, is associated with LVH in diabetic haemodialysis patients.

Autonomic neuropathy is a common complication of diabetes mellitus, and the presence of cardiovascular autonomic neuropathy is associated with adverse cardiac outcome. In a study of 120 diabetic patients who had been followed-up for an average of 4.5 years, major cardiac events were significantly more common in patients with than in those without cardiovascular autonomic neuropathy (24 vs 7%) [3]. In addition, a meta-analysis of studies of diabetic patients concluded that the mortality of autonomic neuropathy-free subjects during 5.5 years of observation was ~5%, but this increased to 27% with onset of cardiovascular autonomic neuropathy [5]. When impairment of cardiovascular autonomic function is combined with LVH, an independent risk factor for shortened survival in ESRD patients [1] as well as for cardiovascular diseases including coronary artery disease or arrhythmias [13–15], the mortality of ESRD patients is increased further. To improve prognosis in diabetic haemodialysis patients, mechanisms linking LVH to cardiovascular autonomic neuropathy should be elucidated.

In diabetic patients, the baroreflex dysfunction due to impairment of the afferent limb of the reflex has been well established [16]. Sinoaortic denervation, which results in baroreflex dysfunction, causes persistent apoptosis in myocardicocytes of rats, although the mechanism has not been clarified [17]. Apoptosis in myocardic cells is involved in cardiac remodelling including LVH in human diabetes [18]. The previous study showed a correlation between diminished baroreflex sensitivity and LVH [19], but the precise relationship between them remains to be clarified [20]. Since the baroreceptor-heart rate reflex dysfunction appears to represent an early stage of cardiovascular autonomic neuropathy as a diabetic complication [21], diabetic haemodialysis patients who had shown impaired parasympathetic activity in the analysis of heart rate variability in the present study were likely to have baroreceptor-heart rate reflex dysfunction. It may make a contribution to understanding the association between the cardiovascular autonomic neuropathy and LVH if we were to investigate the relationship between myocardial apoptosis and impaired parasympathetic activity or baroreflex dysfunction in diabetic haemodialysis patients.

A single haemodialysis treatment reportedly restores the LF component in heart rate variability in non-diabetic ESRD patients [22]. The decrease in blood volume by haemodialysis elicited an increase in heart rate at rest and during sympathetic activation by tilt, and augmented the heart rate response to tilt in patients after dialysis compared with the state before dialysis in non-diabetic ESRD patients [23]. In addition, acute volume expansion of the intrathoracic compartment of the circulation by head-down tilt was able to reduce the overall heart rate and blood pressure variability in healthy subjects [24]. These findings indicate that overhydration may be involved at least partly in reduced heart rate variability before dialysis in non-diabetic ESRD patients, and that the correction of overhydration by ultrafiltration during a haemodialysis treatment is likely to improve the abnormal heart rate variability after dialysis. Because we had carried out Holter ambulatory ECG monitoring for 24 h before a midweek dialysis session, it would be conceivable that overhydration to some extent had affected the results of heart rate variability of non-diabetic haemodialysis patients.

Loss of diurnal variation in blood pressure, with supine hypertension occurring at night, has been noted particularly in diabetic ESRD patients with autonomic neuropathy [25]. In the present study, mean night time systolic and diastolic blood pressures were higher than daytime pressures in diabetic subgroups with or without LVH. On the other hand, mean systolic and diastolic blood pressure did not differ during the daytime or night time between diabetic groups with or without LVH. In contrast, mean systolic and diastolic blood pressures during the night time were higher in non-diabetic patients with LVH than in those without LVH. However, we cannot insist that changes in blood pressure do not seem to be involved in LVH of diabetic haemodialysis patients from the results of the present study. A previous study showed that hypertension was not adequately controlled between dialysis sessions in haemodialysis patients, even though the patients had seemingly ultrafiltration-correctable arterial hypertension [26]. Because ambulatory blood pressure recordings were carried out at 30 min intervals during the daytime and at 60 min intervals at night, it is quite possible that we may have missed the points of high blood pressure during the blood pressure monitoring in patients with LVH. Further investigation would be needed to clarify the precise relationship between hypertension and LVH in diabetic haemodialysis patients.

The results of the present study have shown that cardiovascular autonomic neuropathy may be associated with LVH in diabetic ESRD patients undergoing maintenance haemodialysis, although a cause-and-effect relationship between them is unknown. Coexistence of cardiovascular autonomic neuropathy and LVH, both of which are independent risk factors for major cardiac events such as myocardial infarction or congestive heart failure [1,3–5], is likely to be one of the key factors for poor prognosis in diabetic ESRD.
patients. We need further investigation to clarify the mechanism linking cardiovascular autonomic neuropathy with LVH in diabetic haemodialysis patients.

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Conflict of interest statement. None declared.

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