Cardiovascular Autonomic Neuropathy Studied by a Laser-Doppler Blood Flowmeter in Hemodialysis Patients

Kazuhiro Okano1,2, Yuki Tsuruta1, Tetsuri Yamashita1, Yoshihisa Echida1, Takashi Kabaya2 and Kosaku Nitta1

Abstract

Objective Orthostatic hypotension during a hemodialysis (HD) session affects not only the modality but daily quality of life for HD patients because many of them have combined dysfunction of both sympathetic and parasympathetic nervous systems. Although various non-invasive methods have been applied for the evaluation of autonomic function, no monitor has been devised for measuring the dysfunction during blood purification therapy.

Patients and Methods We evaluated the usefulness of laser-Doppler blood flowmeter (LDF) for measuring autonomic function of stable 34 regular HD patients and 24 healthy controls. The LDF device was applied for autonomic test by measuring periflux blood flow decreasing velocity (PDV) accompanied with Valsalva maneuver. We also evaluated the correlation between PDV and conventional tests for atherosclerosis.

Results The average PDV (3.79 ± 1.77) in HD population level was significantly lower than that of healthy controls (8.72 ± 6.00). We also found a significant correlation between PDV and conventional methods such as heart rate variability and ankle-brachial blood pressure index.

Conclusion Measurement of PDV by LDF is as useful as a conventional method for evaluating autonomic function in HD patients. The convenience of the device offers the benefit of daily and frequent measurement of autonomic dysfunction.

Key words: ABI, PWV, autonomic neuropathy, periflux blood flow, laser-Doppler blood flowmeter

(DOI: 10.2169/internalmedicine.49.3884)

Introduction

Cardiovascular disease is a major cause of mortality in hemodialysis (HD) patients, which may in part be explained by abnormalities in cardiovascular autonomic regulations. Increasing number of HD patients with higher age and diabetes mellitus (DM) stimulates morbidity of autonomic disorder in HD population (1). Quite often, orthostatic hypotension during and after HD session affects not only the modality but the daily quality of life of HD patients (2). Reduced cardiac performance secondary to left ventricular hypertrophy and myocardial interstitial fibrosis has been implicated in the condition (3). And other hypothesis for this complication is autonomic neuropathy caused by uremic poisoning of the baroreceptors (4).

Many groups have reported impairment of cardiac baroreceptor sensitivity in HD patients with intra- or post-dialysis hypotension (5, 6). Converse et al reported that abrupt withdrawal of reflex vasoconstriction is one important cause of hypotension during HD session (7). Standard testing of the integrity of the parasympathetic nervous system include the measurement of the coefficient of variation of R-R intervals (CVRR), the Valsalva maneuver and response to orthostasis (8). Both Valsalva ratio and heart rate responses were reduced in HD or peritoneal dialysis group compared to the normal population (6, 9). These results suggest combined mechanisms of both sympathetic and parasympathetic nerv-
ous dysfunction in uremic conditions. Cardiovascular autonomic dysfunction is closely related to morphological and characteristic alterations of cardiovascular vessels (10-12), but the pathogenesis is still unknown.

Although various non-invasive methods, such as ankle-brachial blood pressure index (ABI), pulse-wave velocity (PWV), and thermography, have been applied for the evaluation of peripheral arterial diseases (PAD), no monitor has been devised during blood purification therapy. Non-invasive continuous monitoring method (NICOMM) of a microcirculation monitor using a laser-Doppler blood flowmeter (LDF) was conducted for studying blood flow during blood purification therapy (13). Furthermore, the LDF device is possible to apply for autonomic test, in which neither exercise nor invasive study is required to participants but perfux blood flow monitoring accompanied with Valsalva maneuver. The system is expected to be useful for measurement of autonomic function for HD patients before or after dialysis session because of a very low amount of load in each experiment.

Since atherosclerosis can be one of causes for autonomic dysfunction in HD population, we enrolled HD patients with stably controlled dialysis therapy and without DM, because insufficient dialysis and DM could worsen autonomic dysfunction. The first aim of this study was to evaluate the usefulness of LDF for measuring autonomic function in regular HD patients. The second aim was to show that blood flow monitoring by NICOMM suggests the severity of atherosclerosis so that it would be useful to evaluate the correlation between the LDF levels and conventional tests. Further, we examined whether anti-platelet drug frequently used against PAD would be beneficial for recovering the LDF levels in HD patients.

Patients and Methods

Participants

Thirty-four stable HD patients (17 females and 17 males, 65.5 ± 15.6 years old) and 24 healthy volunteers (10 females and 14 males, 51.5 ± 16.6 years old) were selected for the study. All of the participants gave their written informed consent to participate the study. The cause of renal failure was renal sclerosis or chronic glomerulonephritis. The patients with DM-induced renal failure were excluded from the study because many have autonomic dysfunction of varying degrees. HD patients had been treated with HD therapy very stably for over 3 months prior to the measurement, using the same membrane and the same dialysis procedure during the study. Hemodynamic stability was defined as a lack of episodes of hypotension during the previous 3 months, with three weekly HD sessions (14) and all the participants selected in this study filled the criteria. Maintenance HD was carried out for 3-4 hour every session, 3 times a week with blood flow rate of 150-220 mL/min, and with dialysate flow rate of 500 mL/min 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). Each subject was requested to refrain from smoking and drinking coffee for at least 12 hours before the test, which was performed early in the morning or afternoon. Aspirin and other nonsteroidal anti-inflammatory drugs were not taken for 1 week before the study. Hypotensive drugs such as angiotensin converting enzyme inhibitor, angiotensin receptor blocker, calcium channel blocker, or β-blocker, were stable during the study. Twenty-seven participants have taken these drugs as hypertension group (HT group) and 7 as non-hypertensive group (non-HT).

Laser-Doppler flowmeter

The room temperature was 24 ± 2°C. During a 30-min acclimatization period, both an earlobe and a finger without arteriovenous-fistula was gently cleaned with ethanol and then allowed to dry. Microvascular blood flow was examined using a LDF, CyberMed® CDF-2000 (Libmeech, Tokyo, Japan), using a 780 nm wavelength, attached to these areas (Fig. 1A). The size of the novel LDF probe is designed to allow the laser irradiator and the detector to be mounted together in the small probe section. The conventional LDF probe does not contain illuminator and detector together inside the probe. The conventional probe is not suitable for a long time measurement because of considerable noise by body movement. However, the illuminator and detector are set at the point of integrated probe so that it minimizes noise and allows prolonged measurement (13-15). The subjects were seated, with the LDF probe affixed to finger or earlobe using double-sided tape.

Quantifying autonomic function in Valsalva maneuver

The participant was instructed in Valsalva’s maneuver in which respiration was held for 10 second at the maximum inspiration, keeping seated posture (Fig. 1B). Periflux blood flow decreasing velocity (PDV) was established for determining autonomic abnormality (16, 17) (Fig. 1C). The experiments were performed at least 3 times just prior to dialysis session at two different days and the mean ± SD was calculated.

Heart rate variability

After measuring PDV, subjects were allowed to lie comfortably upon the treatment bed for a 10 minute equilibration period before recording surface electrocardiogram. CVRR with the minimal SD were automatically extracted from the obtained data (18).

Brachial-ankle PWV and ABI

Brachial-ankle PWV, a parameter for the measurement of arterial stiffness, and ABI were measured using a volume-plethymographic apparatus (PWV/ABI, Colin Co. Ltd., Komaki, Japan) according to methods previously reported (19-21).
Figure 1. (A) Non-invasive continuous monitoring method (NICOMM) system is possible to measure skin tissue contained in a hemisphere with a diameter of 3 to 4 mm from the surface. (B) The participants maintained a seated position during the measurement. (C) Display shows periflux blood flow decreasing velocity (PDV) on the vertical axis and recorded time (sec) on the horizontal axis. The results are expressed in mL/min/100 g./

Laboratory and clinical studies

Hematological and biochemical blood samples were collected after at least 12 hours of fasting just prior to the first HD session of week and tested at the laboratory at Minami-senju hospital. Blood pressure (BP) was measured hourly during HD using a mercury sphygmomanometer. Mean levels of systolic and diastolic BP was determined as the means of measurements obtained in 8 different midweek HD sessions in which patients showed essentially the same increase in body weight (22).

Sarpogrelate hydrochloride study

The participants who gave written informed consent for the experimental protocols were randomized and divided into two groups, in prospective and comparative study. Patients in group A (n=17; 10 men and 7 women; mean age, 62.7 ± 15.6 years old) were orally given sarpogrelate hydrochloride (Mitsubishi Well Pharma Co, Osaka, Japan), a 5HT\textsubscript{2A} receptor blocker, at a dose of 300 mg (100 mg 3 times daily). Patients in group B (n=17; 7 men and 10 women; mean age, 67.2 ± 14.3 years old) were treated with placebo as a control. Each drug was crushed into complete powder, and then poured into undistinguishable capsules. PDV was measured at 0, 1, and 3 months after starting observation. All the patients had no change of medication during the observational period.

Statistical analysis

Two sided t-test or Mann-Whitney U analysis were used to compare parametric and non-parametric data, respectively. The data were expressed as mean values ± SD. Analyses exploring relationship between PDV levels and variables were performed using the Pearson correlation test. A $P$-value of <0.05 was considered to be statistically significant.

Results

First, we compared the PDV levels of regular HD patients to those of healthy controls (Table 1). The average PDV level was 3.79 ± 1.77 in HD populations, while 8.72 ± 6.00 in the control, indicating that the autonomic functions of uremic patients were decreased compared to the healthy controls even if they are treated stably with regular HD ther-
apy (Fig. 2, left panel). The averaged levels of PDV showed no difference between HT and non-HT groups (Fig. 2, right panel).

Next, simple linear regression analysis was performed for the PDV levels against CVRR among the HD population. The PDV significantly showed a positive correlation with CVRR ($R=0.567$, $p<0.001$) (Fig. 3). The result indicates that the PDV is as useful as a conventional parameter for the measurement of autonomic functions in the HD patients.

Since several reports have shown that peripheral arterial stiffness is strongly correlated to the autonomic disregulation (23), we evaluated the relationships of the PDV levels against ABI and PWV. PDV shows significantly positive correlation with ABI (Fig. 4, left panel), but not with PWV (Fig. 4, right panel). PWV analysis is an established diagnostic tool accessing general arterioscleroses of great vesse-

**Table 1. Characteristics of Participants in the Control and HD Groups**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=24)</th>
<th>HD (n=34)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : Female</td>
<td>14:10</td>
<td>16:22</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.1±14.8</td>
<td>59.1±13.3</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>135±19</td>
<td>145±15</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>74±11</td>
<td>76±12</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.9±3.0</td>
<td>19.8±2.4</td>
<td>$p&lt;0.001$</td>
</tr>
</tbody>
</table>

**Laboratory data**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (g/dL)</td>
<td>7.0±0.4</td>
<td>6.9±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Alb (g/dL)</td>
<td>3.9±0.3</td>
<td>3.8±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>UN (mg/dL)</td>
<td>19.8±0.84</td>
<td>67.5±10.3</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.9±0.2</td>
<td>9.7±2.9</td>
<td>$p&lt;0.001$</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>167.1±80.3</td>
<td>151.0±33.8</td>
<td>NS</td>
</tr>
<tr>
<td>HDLc (mg/dL)</td>
<td>35.0±8.0</td>
<td>40.1±10.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means±SD, NS: nonsignificant. $p<0.05$ is considered as significant.


**Figure 2.** (left panel) Mean levels of PDV of 34 hemodialysis patients and 24 healthy controls. (right panel) Mean levels of PDV of 27 or 7 hemodialysis patients with or without hypertension, respectively. Data are mean ± SD.
sels (24) and low scores of ABI show the existence of peripheral arterial disease (25). Our data suggest that parasympathetic dysfunction is strongly associated with peripheral vessel disease rather than large vessels in HD population.

Since atherosclerosis is likely to contribute to the progression of autonomic dysfunction, we examined whether the alteration of the PDV levels are observed when HD patients are categorized with or without oral administration of a 5-HT<sub>2A</sub> receptor blocker for 3 months, which is frequently used for treatment of PAD. The laboratory and clinical data are shown in Table 2 and there was no significant difference between the two groups. We observed no improvement in the PDV levels in the drug-administration group compared to the control group (Fig. 5). The result suggests that short-period treatment with a 5HT<sub>2A</sub> receptor blocker has no recovering effect on autonomic function of regular HD patients at least when it is administered for a relative short period such as 3 months.

**Discussion**

The average age of HD patients continues to increase every year and DM nephropathy is the most common cause for introduction to HD therapy. These result in increasing number of HD patients with autonomic dysfunction during or after HD sessions. However, the dysfunction is difficult for objective and quantitative evaluation so that the severity of the dysfunction is not well understood and difficult to compare among different hospitals in various areas. The study was designed for evaluating usefulness of a new, non-invasive, measurement for autonomic function and to compare the new method with the conventional measurement in HD population. Another aim was to examine the contribution of atherosclerosis on autonomic dysfunction in HD patients without DM of insufficient dialysis.

The mean level of PDV in HD patients is significantly lower than that in healthy controls, indicating that quantitative measurement by LDF is compatible for clinical observation about cardiovascular autonomic deterioration in various dialysis units. The device employed in the study is new so that, as far as we could find, there is no report about the average PDV levels in healthy and CKD population. The average levels of healthy controls vary widely probably due to the relatively small number of participants or to their widely varied backgrounds. However, the number is sufficient to indicate that there is significant difference between the two groups. We consider that the device is useful to compare time-course alteration of autonomic dysfunction in each HD patient and among various dialysis centers in various areas.

Heart rate responses to respiration have been employed for evaluating parasympathetic nervous system function in CKD populations (26). Röckel et al examined alteration of heart rate and blood pressure responses to the Valsalva maneuver in chronic renal failure and reported that 60% of them demonstrated abnormal heart rate responses to the maneuver (9). The new device is not only reliable but also it is handy in measurement at bed-side of a dialysis console, showing that NICOMM is more suitable for both clinical and experimental purposes than conventional methods.

Arterial stiffness has been reported to be an independent
predictor of all-cause mortality in the CKD population (27). Baroreflex sensitivity is correlated with peripheral arterial stiffness in HD patients (23). Brachial-ankle PWV is negatively associated with heart rate variability as an indicator of parasympathetic nervous activity (28). Goernig et al reported that severity of peripheral arterial disease categorized by ABI affects heart rate variability in cardiovascular patients (29). Our results show that PDV has significant, positive correlation with ABI, suggesting that obstruction of arterial vessels affects on autonomic dysfunction in HD patients more than arterial stiffness. Both ABI and PWV reflect alteration of relatively large vessels so that they give little information about small vessels. Our results support a previous report by Goernig et al Complex mechanisms of autonomic neuropathy in uremic patients require further studies.

Since it is likely that atherosclerosis affect on progression of autonomic dysfunction as uremic toxins and diabetes in HD population, sarpogrelate hydrochloride, a selective 5-HT2A antagonist, has widely used for the treatment of PAD (30), leading a question whether the drug improve parasympathetic dysfunction as well as PAD. Activation of central 5-HT2A receptor causes sympathoexcitation in rat (31). Nishizawa et al. showed that sarpogrelate hydrochloride improves chronic pain induced by diabetic neuropathy in patients with type 2 diabetic mellitus (32). Short-term treatment with the drug has no effect probably because of the observational period. Another possibility is that autonomic dysfunction in HD patients is caused by complex diseases such as uremia, hypertension, etc., even though patients with DM or those under dialysis were excluded from the study. Disturbance of microvascular circulation and up-regulated serotonin (5-HT) are considered as underlying mechanisms of diabetic neuropathy (33). Longitudinal administration of the drug is attractive to evaluate its effect on autonomic dysfunction in uremic population. However, long-term observation may require more participants to exclude effects of other factors such as HD condition or medication.

The present experiments demonstrate that measurement of PDV by LDF is as useful as the conventional method for evaluating autonomic function in HD patients. In addition,
the levels of PDV is positively correlated with obstruction of peripheral arterial disease measured by ABI in HD population. The device is so manageable that frequent and simple measurement of PDV gives us beneficial information for better treatment of HD patients.

Acknowledgement
We would like to thank Mr. T. Ohba and Ms. T. Shiraishi for valuable technical assistance.

References