Evaluation of diabetic neuropathy using the tone-entropy analysis, a non-invasive method to estimate the autonomic nervous function

Kenji Karino1*, Toru Nabika2, Masateru Nishiki3, Kenji Iijima4, Atsushi Nagai1,5 and Junichi Masuda5

1 Central Clinical Laboratory, the University Hospital, 2 Department of Functional Pathology, 3 The First and 4 Third Departments of Internal Medicine, 5 Department of Laboratory Medicine, Shimane University School of Medicine, Izumo 693-8501, Japan

(Received 30 October 2008; and accepted 12 November 2008)

ABSTRACT

It is clinically important to diagnose diabetic neuropathy at the early stage. In this study, the tone-entropy analysis of electrocardiogram was applied to diabetic patients to evaluate its usability in the screening of diabetic neuropathy. Consecutive 102 diabetic patients were invited to the study. Electrocardiogram was obtained and analyzed for the tone and the entropy using an original software developed previously. Nerve conduction velocity (NCV) was examined on the median, the posterior tibial and the sural nerves. Patients were divided into quartile subpopulations according to the NCVs in the analysis. Both the tone and the entropy significantly correlated with NCVs, while coefficient of variation in R-R intervals did not show a significant correlation. The correlation was most significant between the entropy and the NCV on the sural nerve. When a multivariate analysis (ordinary regression) was applied to examine independent effects of the factors influencing the NCV on the sural nerve, the entropy was the most potent independent factor ($\beta = 1.14 \pm 0.32$, $P = 0.0004$) along with sex ($\beta = 0.43 \pm 0.19$, $P = 0.02$) and BMI ($\beta = 0.11 \pm 0.05$, $P = 0.04$). The tone-entropy analysis on electrocardiogram may be a promising non-invasive screening method for diabetic neuropathy.

Diabetes mellitus (DM) has been a serious health problem both in developed and developing countries (21, 22). It imposes a heavy burden both on a society and patients themselves due to severe complications such as retinopathy, nephropathy as well as other macro- and microangiopathies (5, 8, 15). Among such complications, peripheral nerve disorders appear at the early stage of DM, and thus its prevalence is high in the patients (4, 8, 15). Further, somatic nerve disorders, when advanced, lead to sensory paralysis and autonomic disturbance such as orthostatic hypotension, dyshidrosis and digestive disfunctions, which frequently influence prognosis and quality of life of the patients (5, 15, 24). To prevent the complications above, it is therefore important to diagnose the diabetic neuropathy at the early stage. The diagnosis is, however, often difficult because the neuropathy progresses latently without distinct symptoms (4, 24).

To diagnose diabetic neuropathy, the peripheral nerve conduction velocity (NCV) is the golden standard (13, 20). However, as NCV requires a skilful technique to obtain accurate data, it is not suitable for a screening examination. Instead of that, the spectral analysis of heart rate has been used in the evaluation of autonomic disorders as a non-invasive method (19, 23). This analysis is based on the observation of reduced physiological variability of heart rate in autonomic disorders (3, 19, 23). The analysis is, however, sometimes argued not to be reliable when respiration rate is out of the normal
range or when inter-individual comparison is attempted (10, 19, 23). In this context, the tone-entropy (TE) analysis was introduced as an alternative method to evaluate the autonomic function (1, 16, 18). In this analysis, the variation of the R-R interval was used to calculate the tone and the entropy as defined previously (2, 18). Oida et al. indicated that the tone was influenced by the autonomic nerve activity while the entropy depended on the balance between the sympathetic and the parasympathetic activity (1, 16). From the clinical point of view, the TE analysis has a potential advantage over the spectral analysis because it enables a direct comparison of the autonomic activity between individuals even under respiratory noises (1, 18).

In the present study, to evaluate the usefulness of the TE analysis in the diagnosis of diabetic neuropathy, we compared the TE analysis with the NCV in patients with DM.

MATERIALS AND METHODS

Subjects. Consecutive diabetic inpatients in Shimane University Hospital were invited to the study between September 2004 and November 2005. Patients with abnormal cardiac rhythm, arterial fibrillation or atrioventricular block were excluded. A hundred and two (46 males and 56 females) participants who gave an informed consent were included in the study. Of them, nineteen (9 males and 10 females) had either nephropathy or retinopathy as diabetic complications. Total cholesterol (T-chol), high density lipoprotein cholesterol (HDL-C), fasting plasma glucose (FPG), and HbA1c were measured after 12 h of fasting. This study was approved by the Ethics Committee of Shimane University School of Medicine.

Physiological measurement. The TE measurement was performed in the morning to avoid the diurnal difference due to the circadian rhythm. The TE data were obtained after at least 10-min rest at the spine position. The electrocardiogram (FPC-4301; Fukuda Denshi Co., Tokyo, Japan) was recorded for 5 min, converted into digital signal at a sampling rate of 10 kHz, and analyzed using a software developed by Oida et al. (1, 18). The tone and the entropy were calculated with the following formulae (18):

\[
\text{Tone} = 1 / N \cdot \sum P(n)
\]

Where, \( P(n) = [H(n) - H(n + 1)] \cdot 100 / H(n); \) \( H(n) \) is the R-R interval between \( nth \) and \( (n - 1)th \) beat.

\[
\text{Entropy} = - \sum_i p(i) \log_2 p(i) \text{ (bit)}
\]

Where, \( p(i) \) is the probability for the events in which \( P(n) \) has a value in a range \( i \leq P(n) \leq i + 1 \).

Coefficient of Variation of R-R intervals (CVRR) was obtained with a software implemented in the electrocardiograph simultaneously when the TE data collection was performed.

Motor nerve conduction velocity (MCV) and sensory nerve conduction velocity (SCV) were measured using an EMG/evoked potential measuring system (Neuropack Λ; Nihon Kohden Co., Tokyo, Japan). MCV and SCV were measured as orthodromic and antidromic conduction, respectively, in the median nerve (Med-MCV, Med-SCV), the posterior tibial nerve (Tib-MCV), and the sural nerve (Sur-SCV). The nerve stimulation was performed for 20–40 ms at the frequency of 1 Hz. The average of 20 measurements was used as a representative value in the study.

The carotid-femoral pulse wave velocity (cfPWV) and the ankle-brachial pressure index (ABI) were measured using a mechanocardiograph (VaSera VS-1000; Fukuda Denshi Co., Tokyo, Japan). The intima-media thickness (IMT) in the carotid artery was evaluated with a sonography equipped with a 7.5 MHz linear probe (ATL HDI 5000; Philips Medical Systems, Netherlands). The maximal IMT (max IMT) was measured at the bilateral common carotid arteries, the carotid bulbs, and the proximal internal carotid arteries.

Statistical analysis. Values were expressed as mean ± standard error of the mean. The subjects were divided into quartile classes according to each of the 4 NCVs; the ranges of the quartile 1 to 4 for Med-MCV are 63.4 to 54.8, 54.8 to 51.8, 51.8 to 47.5 and 47.5 to 40.9 m/s, respectively. In the same way, for Med-SCV; 70.5 to 61.8, 61.8 to 58.5, 58.5 to 52.9 and 52.9 to 34.9 m/s, for Tib-MCV; 50.7 to 44.1, 44.1 to 41.1, 41.1 to 38.1 and 38.1 to < 10 m/s, and for Sur-SCV; 57.2 to 50.9, 50.9 to 46.2, 46.2 to 41.4 and 41.4 to < 10, respectively. All the statistical analyses were performed using JMP v.7 (SAS Institute, Cary, NC).

RESULTS

The tone and the entropy were plotted in Fig. 1. The distribution was similar to the ones reported previously (1, 2, 16, 18). The patients with retinopathy or nephropathy tended to have higher tone and lower
entropy though they were not statistically significant (data not shown). The four NCVs correlated well with each other (data not shown, Speaman’s ρ were between 0.47 and 0.63, \( P < 0.0001 \)). When the patients were divided into 4 classes according to each of the four NCVs (see the panel at the bottom of Fig. 2), the tone and the entropy were significantly associated with Med-SCV, Tib-MCV and Sur-SCV (Fig. 2). Even after the Bonferroni’s correction (the significant level was \( P = 0.004 \)), the tone and the entropy showed significant association with Sur-SCV. On the other hand, neither the tone nor the entropy was significantly associated with Med-MCV though the tendency similar to other NCVs was observed. CVRR was not significantly different among the quartile classes of any NCVs.

As Sur-SCV showed the most potent association with the tone and the entropy, various parameters potentially affecting Sur-SCV were evaluated in a univariate analysis. As indicated in Table 1, the male/female ratio and BMI differed significantly among the quartile classes of Sur-SCV in addition to the tone and the entropy (by the non-parametric Kruskal-Wallis test). The parameters for DM (FPG and HbA1c) or for athero- and arteriosclerosis (ABI, cfPWV and max IMT) did not correlate significantly with Sur-SCV.

Finally, a multivariate evaluation of the parameters contributing to the quartile classification of Sur-SCV was done using the ordinary logistic regression analysis including sex, BMI and the entropy as independent variables. Table 2 summarizes the result; the entropy remained to be the factor associated most strongly with Sur-SCV even after adjustment with sex and BMI. When the tone was substituted for the entropy, a similar result was obtained (data not shown). In addition, similar results were ob-

![Fig. 1](image1.png) The tone and entropy profile for the studied subjects. The measurement of the tone and the entropy was performed as described in Methods. Open circles and triangles indicate the subjects with and without diabetic nephropathy and/or retinopathy, respectively.

![Fig. 2](image2.png) Effects of NCVs on the tone, entropy and CVRR. The subjects were divided into quartile subpopulations according to the four NCVs as described in Methods (see the bottom panel). The tone, entropy and CVRR were compared among the four subpopulations using nonparametric Kruskal-Wallis test. Columns and error bars indicate mean and SE, \( P \) values are shown when they are less than 0.05 except for NCVs (NCVs were significantly different among the quartile subpopulations at \( P < 0.0001 \)). CVRR: Coefficient of Variation of R-R intervals, NCVs: nerve conduction velocities, Med-MCV: motor nerve conduction velocity in the median nerve, Med-SCV: sensory nerve conduction velocity in the median nerve, Tib-MCV: motor nerve conduction velocity in the posterior tibial nerve, Sur-SCV: sensory nerve conduction velocity in the sural nerve.
entropy correlated with all of the four NCVs studied, the correlation with Sur-SCV was the most prominent. As it is commonly believed that reduction in Sur-SCV is one of the earliest signs for diabetic neuropathy (4, 9), this observation further supports the usefulness of the TE analysis.

It is of note that the TE analysis estimates the autonomic nervous function regulating heart rate. Hence it is quite interesting that the entropy, a parameter for the balance between the sympathetic and parasympathetic nerves regulating the heart rate, showed a highly significant correlation with a NCV in a peripheral sensory nerve in the leg, i.e., the sural nerve. As it is believed that diabetic neuropathy first involves both autonomic and sensory nerves, the cardiac autonomic nerves may be one of the earliest targets for diabetes along with the sural nerve (9, 11).

There are two major hypotheses concerning the pathogenesis of diabetic neuropathy: 1) diabetic mi-

Table 1  Demographic data of the studied population stratified with Sur-SCV

<table>
<thead>
<tr>
<th>Quatile 1</th>
<th>Quatile 2</th>
<th>Quatile 3</th>
<th>Quatile 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (M/F)</td>
<td>24 (18/6)</td>
<td>26 (11/15)</td>
<td>25 (9/16)</td>
</tr>
<tr>
<td>Age, years</td>
<td>61.2 ± 2.4</td>
<td>63.8 ± 2.3</td>
<td>62.8 ± 2.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.4 ± 0.8</td>
<td>22.7 ± 0.7</td>
<td>21.4 ± 0.8</td>
</tr>
<tr>
<td>MBP, mmHg</td>
<td>94.4 ± 2.4</td>
<td>87.9 ± 2.3</td>
<td>93.1 ± 2.4</td>
</tr>
<tr>
<td>T-chol, mg/dL</td>
<td>195 ± 9</td>
<td>177 ± 9</td>
<td>190 ± 9</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>54.0 ± 3.8</td>
<td>50.0 ± 3.6</td>
<td>55.3 ± 3.7</td>
</tr>
<tr>
<td>FPG, mg/dL</td>
<td>154 ± 11</td>
<td>146 ± 11</td>
<td>161 ± 11</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.8 ± 0.4</td>
<td>7.6 ± 0.4</td>
<td>8.1 ± 0.4</td>
</tr>
<tr>
<td>ABI</td>
<td>1.16 ± 0.02</td>
<td>1.12 ± 0.02</td>
<td>1.13 ± 0.02</td>
</tr>
<tr>
<td>cpPWV, m/s</td>
<td>7.59 ± 0.50</td>
<td>7.88 ± 0.48</td>
<td>8.83 ± 0.49</td>
</tr>
<tr>
<td>max IMT, mm</td>
<td>1.94 ± 0.25</td>
<td>2.05 ± 0.28</td>
<td>2.08 ± 0.28</td>
</tr>
<tr>
<td>Tone</td>
<td>−0.027 ± 0.005</td>
<td>−0.024 ± 0.004</td>
<td>−0.007 ± 0.005</td>
</tr>
<tr>
<td>Entropy, bit</td>
<td>2.87 ± 0.12</td>
<td>2.65 ± 0.11</td>
<td>2.46 ± 0.12</td>
</tr>
<tr>
<td>CVRR, %</td>
<td>2.37 ± 0.24</td>
<td>2.54 ± 0.26</td>
<td>1.97 ± 0.24</td>
</tr>
<tr>
<td>Sur-SCV, m/s</td>
<td>53.4 ± 1.5</td>
<td>48.2 ± 1.4</td>
<td>44.3 ± 1.5</td>
</tr>
</tbody>
</table>

*: by Kruskal-Wallis test or χ² test
BMI: body mass index, MBP: mean blood pressure, T-chol: total cholesterol, HDL-C: high-density lipoprotein cholesterol, FPG: fasting plasma glucose, ABI: ankle-brachial index, PWV: pulse-wave velocity, IMT: intima-media thickness, Su-SCV: sural nerve sensory conduction velocity

Table 2  Parameters independently correlated with the Sur-SCV by the ordinary logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>Wald</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.43</td>
<td>0.19</td>
<td>5.08</td>
<td>1.53</td>
<td>1.05–5.99</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI</td>
<td>0.11</td>
<td>0.052</td>
<td>4.15</td>
<td>1.12</td>
<td>1.01–1.23</td>
<td>0.04</td>
</tr>
<tr>
<td>Entropy</td>
<td>1.14</td>
<td>0.32</td>
<td>12.4</td>
<td>3.13</td>
<td>1.71–5.99</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Subjects were divided into quartile classes according to the Sur-SCV. Age, FPG, HbA1c and MBP were excluded by the analysis. Wald: the Wald statistics for the logistic analysis, OR: odds ratio, CI: confidence interval.

served when Med-MCV, Med-SCV or Tib-MCV was substituted for Sur-SCV though the significant level was much lower ($P = 0.05, 0.01$ and $0.004$, respectively).

The analysis was repeated on the participants without diabetic retinopathy and nephropathy as well. The result was similar to that indicated in Table 2; the entropy ($\beta = 1.14 ± 0.39, P = 0.003$) and the sex ($\beta = 0.99 ± 0.46, P = 0.03$) were the two independent factors contributing to the quartile classification although BMI ($\beta = 0.10 ± 0.06, P = 0.09$) did not reach a significant level.

**DISCUSSION**

In this communication, we showed that the TE analysis was useful in the evaluation of diabetic neuropathy. As the TE analysis uses electrocardiographic data, it is simple and non-invasive, which is a great advantage as a screening examination. Although the entropy correlated with all of the four NCVs studied, the correlation with Sur-SCV was the most prominent. As it is commonly believed that reduction in Sur-SCV is one of the earliest signs for diabetic neuropathy (4, 9), this observation further supports the usefulness of the TE analysis.

It is of note that the TE analysis estimates the autonomic nervous function regulating heart rate. Hence it is quite interesting that the entropy, a parameter for the balance between the sympathetic and parasympathetic nerves regulating the heart rate, showed a highly significant correlation with a NCV in a peripheral sensory nerve in the leg, i.e., the sural nerve. As it is believed that diabetic neuropathy first involves both autonomic and sensory nerves, the cardiac autonomic nerves may be one of the earliest targets for diabetes along with the sural nerve (9, 11).

There are two major hypotheses concerning the pathogenesis of diabetic neuropathy: 1) diabetic mi-
Dr. Eiichi Oida in this study. We deeply appreciate the generous cooperation of
Acknowledgement

Diabetic neuropathy sometimes causes severe complications such as autonomic dysfunctions and sensory loss (4, 6, 27). It is thus beneficial for the patients to diagnose it at the earlier stage. Reduction in the entropy and the simultaneous increase in the tone were observed in the elderly (16) and in diabetic patients (17). Repeated use of the TE analysis may aid the diabetic patients to impede the progress of the neuropathy. Larger clinical studies to evaluate the potential usefulness of the TE analysis are warranted.

Acknowledgement
We deeply appreciate the generous cooperation of Dr. Eiichi Oida in this study.

REFERENCES


