Assessment of Diabetic Autonomic Neuropathy using Twenty-Four-Hour Spectral Analysis of Heart Rate Variability

A Comparison with the Findings of the Ewing Battery

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SUMMARY

A power spectral analysis of heart rate variability has been applied in order to assess diabetic autonomic neuropathy and high frequency spectra are thus considered to possibly reflect vagal nerve integrity in patients with diabetes mellitus. The purpose of this study was to investigate the relationship between the findings of high frequency spectra analysis and the results of the Ewing battery.

We performed 24-hour power spectral analysis using an ambulatory ECG monitoring system and standard tests in order to assess diabetic autonomic neuropathy (Ewing battery) in 18 diabetic patients to compare their diagnostic values for diabetic autonomic neuropathy. We used the high frequency amplitude (high frequency spectra; 0.15-0.40 Hz) as a direct measure of vagal nerve integrity from each hourly spectral plot. All hourly high frequency spectra decreased along with the impaired assessment of the battery, especially during the night when the high frequency spectra showed a manifest increase in patients classified as normal according to the battery. High frequency spectra during the night while asleep (22:00-05:00) and during a 24-hour period significantly correlated with the results of the battery. These values markedly decreased even in patients classified as having early vagal damage when compared with those classified as normal. High frequency spectra during night closely reflected the intrinsic vagal nerve integrity in patients with diabetes mellitus.

High frequency spectra during night or a 24-hour period is a simple and sensitive measure of diabetic autonomic neuropathy and is considered to be a useful modality for detecting even early changes in autonomic dysfunction.
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Key words: Heart rate, Autonomic nervous system, Diabetes mellitus

DIABETIC autonomic neuropathy has a wide range of clinical presentations. Since the morbidity and mortality associated with diabetic autonomic neuropathy

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are substantial,^{1,2)} the need for a reliable and sensitive measure of autonomic nerve activity has been stressed. Ewing, *et al*³⁾ introduced a test battery for studying autonomic damage in diabetic patients (Ewing battery). This battery has been widely accepted as a means to classify autonomic neuropathy in terms of its severity, but it has also been criticized since it requires active patient participation, is time consuming, and sometimes difficult to standardize.

A spectral analysis of heart rate variability (HRV) has been frequently utilized to evaluate diabetic autonomic neuropathy.⁴⁻⁶⁾ Akselrod, *et al*⁷⁾ reported that the spectral analysis of HRV possesses three major components shown to be related with different patterns of neural control to the heart, namely; low frequency (0.02-0.09 Hz), mid frequency (0.09-0.15 Hz) and high frequency (0.15-0.40 Hz) components. The power within the high frequency component is widely considered to reflect only vagal nerve integrity, although the power within low frequency and mid frequency components reflects both sympathetic and parasympathetic systems in an ambulatory state, and it is partially influenced by other neurohumoral activities (e.g., renin-angiotensin system). It can also provide an opportunity for assessing the natural activation of parasympathetic nerves during sleep which comprises some of the most basal states of the day. We routinely obtain high frequency spectra in patients during their usual daily activities by means of 24-hour ambulatory ECG monitoring.

Earlier studies show that silent myocardial ischemia as detected by ambulatory ECG monitoring is frequently observed in patients with diabetes mellitus. These observations have now led physicians to use ambulatory ECG monitoring more frequently than before.⁸⁾ According to the increasing utility of ambulatory ECG monitoring in diabetic patients, it is now possible to evaluate HRV more easily together while also identifying silent myocardial ischemic episodes.

Although spectral analysis of HRV has been widely studied, there have only been two reports^{9,10)} to directly compare the high frequency spectra derived from ambulatory ECG monitoring with the traditional Ewing battery. Since it is possible that high frequency spectra reflects purely vagal nerve integrity in patients with diabetes mellitus, we focused our attention on the high frequency spectra in a spectral analysis of HRV. The purpose of this study was thus to investigate the relationship between high frequency spectra and the results of the Ewing battery in order to confirm the usefulness of high frequency spectra measurements in patients with diabetes mellitus.

METHOD

Study populations: Eighteen male non-insulin dependent diabetic patients underwent standard autonomic function tests (Ewing battery) and a 24-hour

power spectral analysis of HRV. Their mean age was 41.1 ± 15.0 years and the duration of diabetes was 5.6 ± 3.9 years. None of the subjects had hypertension or any clinical evidence of heart disease, and none was taking any medication which is known to affect the autonomic nervous system. None had shown any ECG abnormalities on a resting ECG which would prevent them from undergoing measurements of ischemic ST- or T-wave changes and RR intervals (e.g., bundle-branch block, Wolf Parkinson-White syndrome, findings for left ventricular hypertrophy).

Standard autonomic tests (Ewing battery): During the standard tests, blood pressure and heart rate were measured with a non-invasive vital sign monitor (1598-E130, Toshiba Inc. Tokyo, Japan) which has a microcomputer and displays all data in real time. The patients were all given sufficient time to reach their baseline autonomic function levels.

The autonomic battery to assess cardiovascular function consists of three tests reflecting cardiac parasympathetic damage and two tests implying cardiovascular sympathetic damage as follows; 1) the heart rate response to forced breathing, 2) the heart rate response to the Valsalva maneuver, 3) the heart rate response to standing up, 4) the blood pressure response to standing, and 5) the blood pressure response to handgrip.³⁾ We classified the diabetic subjects into one of the four groups according to Ewing's criteria³⁾ no abnormal results (group 1), with one of three tests showing abnormal findings for the parasympathetic function (group 2; early parasympathetic damage), showing abnormal findings for at least two of three tests for parasympathetic function (group 3; definite parasympathetic damage), and showing abnormal findings for one or two tests for sympathetic function in addition to abnormal parasympathetic test results (group 4; combined parasympathetic and sympathetic damage).

Spectral analysis: Two channel recordings were obtained using a Marquette 8500 recorder and were analyzed by the Marquette 8000 system (Marquette Electronics, Milwaukee, WI, USA) which consisted of a computer with a manual overread. HRV was measured using a previously reported method¹¹⁾ and a list of the RR intervals was input into the computer program (Marquette Electronics, HRV Software Version 002A) which allowed spectral analysis of the heart rate using the fast Fourier transform method based on a windowed periodogram technique. A spectral plot for one hour was obtained by averaging spectra derived from a 256 point data series at 2-minute intervals over a one-hour period. Spectral amplitude which is a square root of the area under a power spectrum was used as a spectral measure (ms) instead of power (ms²). We used the spectral amplitude of high frequency spectra as a direct index of parasympathetic nerve function. If the analysis of ambulatory ECG monitoring revealed more than 2% artifact or arrhythmic beats per total beats, the tapes were to be excluded from the analysis.

However, in the present study none of the recorded tapes met this exclusion criterion.

We examined the mean values of high frequency spectra for an entire 24-hour period during the daytime (10:00-17:00), and during the night (22:00-05:00). We chose this night time period because all of the patients were in bed asleep during this period according to their self-recorded diaries.

Statistical analysis: All values are expressed as the mean $\pm$ SD unless otherwise indicated. A one-way analysis of variance (ANOVA) was used to compare high frequency spectra derived from the 24-hour spectral analysis and from the results of the battery in each group, and subsequent alpha levels were corrected using the Scheffe method. Spearman's correlation coefficients were used for the correlation between the high frequency spectra during the indicated time periods. Frequency distributions were compared using the Chi-square test or Fisher's exact test. Differences were considered significant if $p < 0.05$.

RESULTS

Six diabetic subjects had normal autonomic function test results (group 1). Early vagal nerve damage (group 2) was detected in five diabetic patients and four had definite vagal nerve damage (group 3). Sympathetic neuropathy in addition to vagal nerve damage (group 4) was detected in three patients. No differences in age, duration of diabetes, or serum levels of HbA1c (hemoglobin A1c, a glycosylated hemoglobin) were found among the four groups. None of the patients in this study had any silent myocardial episodes during the 24-hour ambulatory ECG monitoring period.

Figure 1 shows the 24-hour variation in the hourly mean high frequency spectra in each group. In group 1, high frequency spectra indicated a distinct 24-hour oscillation which showed a manifest peak at night during sleep and relatively low values during the daytime. In contrast, a reduced 24-hour variation was observed in groups 3 and 4. However, almost all of the hourly plots of high frequency spectra tended to be higher in group 3 than in group 4. In group 2, high frequency spectra showed intermediate values between group 1 and group 3 during the day and also showed a substantial increase during the night. This peak decreased along with the severity of the impairment as assessed by the Ewing battery. The hourly high frequency spectra during night also decreased according to the severity of the Ewing battery findings.

Table I shows the mean values of the high frequency spectra during different time periods for the four groups of diabetic patients. An analysis of variance showed a good separation among the four groups with high frequency spectra during the night time period, and also high frequency spectra during the 24-hour

Figure 1. Diurnal changes in high frequency spectra in each group classified by the Ewing battery. Each point represents the mean value of high frequency spectra every hour. Open square, group 1; Open square with a dot inside, group 2; Closed triangle, group 3; Closed square, group 4. In group 1, the high frequency spectra exhibited a distinct 24-hour oscillation. A reduced 24-hour variation was observed in groups 3 and 4. In group 2, the high frequency spectra showed intermediate values which were between those of groups 1 and 3.

period. The 'F' ratio reflects the overall differences between the groups as classified by the battery and high frequency spectra during the indicated time periods. High frequency spectra during night time sleep (22:00-05:00) showed the highest F ratio. After correcting the statistical alpha levels using the Scheffe method, the mean high frequency spectra values during night could be distinguished among the four groups (Table I). The high frequency spectra values in group 4 were the lowest, while those in group 3 were the second lowest, and those in group 2 were lower than those in group 1 (all significant). These tendencies were also observed in the mean values of high frequency spectra during the 24-hour period.

Figure 2 shows the correlation between the high frequency spectra during different time periods of the day. The high frequency spectra during the 24-hour period showed an excellent correlation ($r=0.97$) with that during the night. The correlation coefficient between the high frequency spectra during the daytime and during the 24-hour period was 0.67, which was significantly smaller than that between the high frequency spectra during the night and a 24-hour period.

Table I. Comparison of the High Frequency Spectra during Different Time Periods in Diabetic Patients

	Group 1 (n=6)	Group 2 (n=5)	Group 3 (n=4)	Group 4 (n=3)	F ratio	p value
HFS from (00:00-24:00)	14.0±2.6	8.7±5.4*	5.3±2.5#	4.6±1.5#	5.326	0.013
HFS from (10:00-17:00)	9.0±3.1	7.4±5.3	5.2±2.6	4.5±1.3	1.868	0.185
HFS from (22:00-05:00)	19.6±3.6	11.6±7.1*	5.7±2.3#†	4.6±1.4#¶	8.187	0.003

(mean ± SD; HFS=high frequency spectra; ms; *p<0.05 vs group 1, # p<0.01 vs group 1, † p<0.05 vs group 1 and group 2, ¶ p<0.05 vs group 1, group 2 and group 3)

Figure 2. Relationship between the high frequency spectra during an entire 24 hour period with those during either the night or daytime. **A:** Relationship between the high frequency spectra during an entire 24 hour period with those during the night. **B:** Relationship between the high frequency spectra during an entire 24 hour period with those during the daytime.

DISCUSSION

The results of the present study demonstrate that the high frequency spectra derived from 24-hour ambulatory ECG monitoring correlates significantly with the results of the Ewing battery, and thus suggest that high frequency spectra might be useful in identifying early autonomic dysfunction in patients with diabetes mellitus. The severity of the autonomic dysfunction determined by the Ewing battery (groups 1 to 4) was correlated significantly with decreases in the high frequency spectra during the night (22:00-05:00). This same tendency was also observed in high frequency spectra during a 24-hour period (00:00-24:00) (Table).

The high frequency spectra during a 24-hour period and during the night correlated significantly with the assessment by the battery and both were significantly decreased in patients classified as having early parasympathetic damage in comparison to those classified as normal. It is not likely that a variety of individ-

ual physical activity caused this reduction, because all of the subjects reported being in bed from 22:00 to 05:00. Since rapid eye movement (REM) sleep, during which sympathetic nerve activity increases and, as a result, parasympathetic nerve activity decreases, is less frequent in diabetic patients,¹²⁾ this reduced parasympathetic tone is probably not caused by an increase in REM sleep. Hourly high frequency spectra during the night appear to closely reflect the intrinsic activity of the vagal nerve in this study.

The findings of the present study suggest that the power spectral indices of HRV during a 24-hour period appear to reflect intrinsic autonomic function although a variety of physical activities may partially mask it during the daytime. It is noteworthy that high frequency spectra during a 24-hour period closely correlated with those observed during the night ($r=0.97$) and also closely correlated with the battery findings. Although we did not evaluate other HRV indices in the present study, other indices of non-spectral analysis of HRV during a 24-hour period such as the proportion of adjacent RR intervals more than 50 ms different (pNN50), the standard deviation of the 5-min mean RR interval (SDNN index), and the root-mean square of the difference of successive RR intervals (rMSSD) are reported to closely correlate with the high frequency spectra during a 24-hour period (pNN50, $r=0.92$; SDNN index, $r=0.90$; rMSSD, $r=0.98$).^{13,14)} Furthermore, parasympathetic nerve activity decreases during the daytime even when lying in bed during the day due to the natural diurnal variation of autonomic nerve activity.¹⁵⁾

Conventional tests^{3,9,16-19)} have usually been performed in the daytime when the activity of the vagal nerve system is relatively reduced, and thus such findings are greatly influenced by several artificial excitations. These tests such as the Ewing battery also require active participation by the patient and improper performance or inadequate effort may also affect the results. As a result, in this regard, it is highly possible that the natural activity during sleep more accurately reveals the actual vagal nerve condition, primarily because the investigator's techniques and/or cooperation of the patient do not affect the results.

The following earlier observations all correlated with our results. Freeman, *et al*²⁰⁾ assessed autonomic integrity by a 3-min power spectral analysis of the resting heart rate in insulin-dependent diabetic patients with symptoms of autonomic dysfunction and reported its clinical usefulness for assessing diabetic autonomic neuropathy. Howorka, *et al*²¹⁾ reported that short-term heart rate spectral analysis showed a similar diagnostic value concerning cardiac autonomic neuropathy as the classical Ewing battery. In addition, Laederach-Hofmann, *et al*²²⁾ recently reported a spectral analysis of HRV to accurately diagnose the early changes in autonomic dysfunction in diabetic patients. Recent advances in the technology of ambulatory ECG monitoring systems have led to the establishment

of a 24-hour spectral analysis of HRV and several reports concerning autonomic integrity assessed by this method have been already published in cardiac disease patients.^{23,24)} The assessment of high frequency spectra obtained from 24-hour ambulatory ECG monitoring is unique for examining the autonomic integrity of natural activity without any artificial excitation.

In the present study, we assessed the amplitude (root square of the power) within the high frequency band as a measure of the parasympathetic nerve system and compared the high frequency spectra findings with those from the battery in order to determine the clinical usefulness of this relatively new method to assess diabetic autonomic neuropathy. Three main peaks in the power spectrum of HRV have been reported,⁷⁾ and an HRV above 0.1 Hz is now generally considered to reflect only parasympathetic nerve integrity. High frequency spectra below 0.1 Hz, however, reflect both the sympathetic and parasympathetic nervous systems and the clinical significance of low frequency and mid frequency components remain a matter of debate, especially when the measurements are performed in an ambulatory state.

Advanced microprocessor technology has now made it easy for clinicians to perform a power spectral analysis of HRV and high frequency spectra values from ambulatory ECG monitoring can be readily obtained. An investigator can analyze data whenever he or she likes and need not go to the patient to perform the tests. Furthermore, using a recently developed ambulatory ECG monitoring system, power spectral analysis needs only slightly more time than a routine analysis of 24-hour ambulatory ECG monitoring to obtain a high frequency spectra. In this respect, the use of high frequency spectra recorded either during the night or during a 24-hour period might not only be more accurate, but may also be a simpler and more practical approach for assessing diabetic autonomic neuropathy. High frequency spectra obtained from a 24-hour ambulatory ECG monitoring system are believed to be particularly useful for detecting early changes regarding autonomic dysfunction in patients with diabetes mellitus.

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