Sympathetic Skin Response and Heart Rate Variability as Differential Diagnostic Tools for Dementia: A Diagnostic Test Study

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2012-001796</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>23-Aug-2012</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Negami, Masako; Keiju Medical Center, Health Service Center Maruta, Takahiro; Kanazawa-Nishi Hospital, Neurological Center Takeda, Chie; Kanazawa-Nishi Hospital, Neurological Center Adachi, Yumi; Kanazawa University, Health Service Center Yoshikawa, Hiroaki; Kanazawa University, Health Service Center</td>
</tr>
<tr>
<td>&lt;b&gt;Primary Subject Heading&lt;/b&gt;:</td>
<td>Neurology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Diagnostics, Neurology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Dementia &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, CLINICAL PHYSIOLOGY, GERIATRIC MEDICINE, Neurology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
Sympathetic Skin Response and Heart Rate Variability as Differential Diagnostic Tools for Dementia: A Diagnostic Test Study

Masako Negami, MD\textsuperscript{1,2}, Takahiro Maruta, MD, PhD\textsuperscript{1,3}, Chie Takeda\textsuperscript{3}, Yumi Adachi, MA\textsuperscript{1}, and Hiroaki Yoshikawa, MD, PhD\textsuperscript{1}

\textsuperscript{1}Health Service Center, Kanazawa University
\textsuperscript{2}Health Service Center, Keiju Medical Center
\textsuperscript{3}Neurological Center, Kanazawa-Nishi Hospital

Corresponding author:
Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
SSR and HRV for Diagnosis of Dementia -2-

Article Summary

Article focus

- To investigate the utilization of the sympathetic skin response (SSR) and the heart rate variability (HRV) for the differential diagnosis of dementia with Lewy bodies (DLB) from Alzheimer’s disease (AD).

Key messages

- The SSR and the HRV could detect the autonomic function as abnormal in patients with DLB at the sensitivities of 85.0% and 90.0%, respectively, on the other hand in patients with AD, the sensitivities were 15.0% and 25% respectively.
- The double-abnormal using combination the SSR and the HRV was only 5% of AD, on the other hand it was 75% of DLB.

Strengths and limitations of this study

- The SSR and the HRV could be useful diagnostic tools for dementia.
- There is 5% of false positive by our methods in patients with AD.
Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study

Setting: Single centre in Japan

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method (MEM).
Results: SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 25% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

Conclusions: These results suggest that SSR and HRV could be useful for differential diagnosis of DLB.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
SSR and HRV for Diagnosis of Dementia -5-

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD).\(^1\) Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, (123)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for DLB.\(^2\) The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. We tested 20 patients with probable AD diagnosed with NINCDS-ADRDA criteria\(^3\) and 20 with probable DLB diagnosed with the criteria of the 3\(^{rd}\) international DLB workshop\(^2\) (Table). There were no differences in MMSE and FAB scores between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50.
Testing of autonomic functions

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. Three waves were recorded for each side (right and left). The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used and a value under 1.01 mV (mean–SD of AD patients) was defined as abnormal.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For recording and analyses of the A-A intervals, an Artett acceleration plethysmography system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method (MEM). The mean of four values, 2 from FFT and 2 from MEM, was used. A value under 1.15 (mean–SD of AD cases) was defined as abnormal. The coefficient of variation of A-A intervals (CVAA) was also analyzed.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized.
SSR and HRV were utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures.

Result

Although there was no difference in the data of the CVAA between patients with DLB and AD, the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HRV (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HRV (LF/HF). For detection of DLB, SSR and HRV had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 78.3%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HRV, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HRV, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an
alternative examination, we studied the possibility of utilizing SSR and HRV.

SSR reflects sympathetic sweat response.\textsuperscript{6} Although SSR amplitude was found to be severely reduced in DLB, there have been no reports of comparison of the data of SSR from DLB and AD.\textsuperscript{7} In this study, the SSR amplitudes in patients with DLB were smaller than those with AD. HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography.\textsuperscript{4} In a previous study,\textsuperscript{8} there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination of sympathetic function.\textsuperscript{9} From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan.

From this study, we recommend the utilization of SSR and HRV in routine clinical practice in patients with dementia.
Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253).

**Funding Statement:** Funded by the Ministry of Health, Labour and Welfare of Japan.

**Contributorship Statement:** Dr. Negami and Dr. Maruta contributed to conception, design, acquisition of data and drafting the article. Dr. Takeda contributed to acquisition of data. Dr. Adachi contributed to analysis and interpretation of data. Dr. Yoshikawa contributed to conception, design and revising article critically for important intellectual content.

**Data Sharing Statement:** We are willing to share additional unpublished data from the study on request.

**Competing Interests Statement:** There are no competing interests.
SSR and HRV for Diagnosis of Dementia -10-

References


Figure legends

Figure  Autonomic examinations.


The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of an Alzheimer disease’s (AD) patient. The SSR of DLB showed almost no response after the electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

2. The X-Y plotting of SSR and the heart to mediastinum uptake ratio (H/M) of the (123)I-metaiodobenzylguanidine (MIBG) scan.

The values of SSR were considered as abnormal when they were smaller than the mean-standard deviation (SD) of patients with AD (dotted line, y=1.01 mV). The values of DLB patients (0.72 ± 0.84; mean ± SD) were smaller than those of AD patients (2.33±1.32; mean ± SD).

3. The X-Y plotting of heart rate variability (HRV) and (H/M) of MIBG scan.

The values of HRV were considered as abnormal when they were smaller than the mean-SD of patients with AD (dotted line, y=1.13). The values of DLB (0.73 ± 0.51; mean ± SD) were smaller than those of AD (2.26±1.11; mean ± SD).

4. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) and the MIBG (H/M).

There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44). The dotted line is the mean + SD of the CVAA in patients with AD (y=5.3).
The values of MIBG (H/M) in patients with DLB were smaller than 1.50 and those of AD were larger than 1.70. P-values were calculated using Wilcoxon’s test.
Table. Comparison of DLB with AD

<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10: male, 10: female</td>
<td>10: male, 10: female</td>
</tr>
<tr>
<td>Age</td>
<td>78.7±6.9* (mean ±SD)</td>
<td>78.5±5.0 (mean ±SD)</td>
</tr>
<tr>
<td>MMSE</td>
<td>19.2±4.8† (mean ±SD)</td>
<td>19.3±3.6 (mean ±SD)</td>
</tr>
<tr>
<td>FAB</td>
<td>8.5±4.2* (mean ±SD)</td>
<td>8.6±3.9 (mean ±SD)</td>
</tr>
<tr>
<td>H/M of MIBG</td>
<td>1.20±0.16‡ (mean ±SD)</td>
<td>1.85±0.15 (mean ±SD)</td>
</tr>
<tr>
<td></td>
<td>(range: 0.95-1.46)</td>
<td>(range: 1.71-2.18)</td>
</tr>
<tr>
<td>SSR abnormal (&gt;1.01)</td>
<td>17/20 § (85%)</td>
<td>3/20 (15%)</td>
</tr>
<tr>
<td>HRV abnormal (&gt;1.15)</td>
<td>18/20 § (90%)</td>
<td>5/20 (25%)</td>
</tr>
</tbody>
</table>

DLB: dementia with Lewy bodies
AD: Alzheimer’s disease
MMSE: mini mental scale examination
FAB: frontal assessment battery
H/M: ratio of heart to mediastinum uptake
MIBG: iodine-123-metaiodobenzylguanidine imaging
SSR: sympathetic skin response
HRV: heart rate variability

* No difference between DLB and AD (Student’s t-test)
† No difference between DLB and AD (Wilcoxon’s test)
‡ p<0.05 between DLB and AD (Wilcoxon’s test)
§ p<0.05 between DLB and AD (Fisher’s exact test)
**Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease**

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2012-001796.R1</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>29-Oct-2012</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Negami, Masako; Keiju Medical Center, Health Service Center Maruta, Takahiro; Kanazawa-Nishi Hospital, Neurological Center Takeda, Chie; Kanazawa-Nishi Hospital, Neurological Center Adachi, Yumi; Kanazawa University, Health Service Center Yoshikawa, Hiroaki; Kanazawa University, Health Service Center</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Neurology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Diagnostics, Neurology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Dementia &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, CLINICAL PHYSIOLOGY, GERIATRIC MEDICINE, Neurology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
SSR and HRV for Diagnosis of Dementia -1-

**Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease**

Masako Negami, MD\(^1,^2\), Takahiro Maruta, MD, PhD\(^1,^3\), Chie Takeda\(^3\), Yumi Adachi, MA\(^1\), and Hiroaki Yoshikawa, MD, PhD\(^1\)

\(^1\)Health Service Center, Kanazawa University

\(^2\)Health Service Center, Keiju Medical Center

\(^3\)Neurological Center, Kanazawa-Nishi Hospital

Corresponding author:

Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
SSR and HRV for Diagnosis of Dementia -2-

Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study

Setting: Single centre in Japan

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM).
Results: SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 15.0% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

Conclusions: These results suggest that SSR and HRV could be useful for differential diagnosis of DLB.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD). Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, (123)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for DLB. The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained by the written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop (Table). To detect the difference of value of 1 with the desired significance level of 0.05 and the power of 0.80, 20-patients in each group was required. There were no differences in MMSE and FAB scores
between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded the patients who could not show the will of participating in this study.

Testing of autonomic functions

Examinations were performed in quiet room in which patients made themselves relax and comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. The waveforms of SSR appeared 1.5 to 2.5-second after the stimulations. Three waves were recorded for each side (right and left). The filters used in measurement were as follows: High cut; 1KHz, Low cut; 0.1Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used for analysis.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For recording and analyses of the A-A intervals, an Artet acceleration plethysmography
SSR and HRV for Diagnosis of Dementia -6-

system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM). The mean of two values from MEM was used. Cut-off value was obtained from ROC curve using DLB as positive level and AD as negative. The coefficient of variation of A-A intervals (CVAA) was also analyzed.

The examinations were performed by a clinical technician with evaluated expertise and the results was reviewed by a special neurologist trained in the electrophysiological laboratory. The technician was blind to the clinical informations of patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curve of the data was drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). The summary of ROC curves in this study was submitted as a supplemental information.

Result

There was no adverse events from performing the examinations. Although there was no difference in the data of the CVAA between patients with DLB and AD,
the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). The cut-off value for SSR was settled at 0.90 mV and that of HVR was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HVR (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HVR (LF/HF). For detection of DLB, SSR and HVR had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HVR, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HVR, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HVR (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for detection of sympathetic activity and is utilized to distinguish DLB from AD.\(^5\) Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an alternative examination, we studied the possibility of utilizing SSR and HVR.

SSR reflects sympathetic sweat response.\(^6\) Although SSR amplitude was found to be severely reduced in DLB, there have been no reports of comparison of the data of SSR from DLB and AD.\(^7\) In this study, the SSR amplitudes in patients with
DLB were smaller than those with AD. HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography.\textsuperscript{4} In a previous study,\textsuperscript{8} there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination of sympathetic function.\textsuperscript{9} From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan. The 3 AD patients with abnormal SSR did not have abnormal MIBG scan. As well, 3 AD patients with abnormal HRV did not have abnormal MIBG scan. Only one AD patient had both abnormal SSR and HRV. We should follow these patients for their clinical manifestations.

From this study, we recommend the utilization of SSR and HRV in routine clinical practice in patients with dementia.
Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253).
References


Figure legends

Figure 1. Autonomic examinations.

A. Records of sympathetic skin response (SSR).

The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of an Alzheimer disease’s (AD) patient. The SSR of DLB showed almost no response after the electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

B. The X-Y plotting of SSR of the patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.90 mV.

C. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.933.

D. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.

There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10 M and 10 F</td>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>78.7±6.9</td>
<td>78.5±5.0</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>19.2±4.8</td>
<td>19.3±3.6</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
<td>8.5±4.2</td>
<td>8.6±3.9</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD) (range)</td>
<td>1.20±0.16 (0.95-1.46)</td>
<td>1.85±0.15 (1.71-2.18)</td>
</tr>
<tr>
<td>SSR (mean ± SD) (Cut-off; 0.90mV) (sensitivity; 85.0%) (specificity; 85.0%)</td>
<td>0.72±0.82*</td>
<td>2.33±1.32</td>
</tr>
<tr>
<td>HRV (mean ± SD) (Cut-off; 0.933) (sensitivity; 90.0%) (specificity; 85.0%)</td>
<td>0.597±0.524</td>
<td>2.276±1.313</td>
</tr>
</tbody>
</table>

*p<0.01 compared with DLB and AD
Fisher's exact probability test was used for analysis.
AD; Alzheimer disease
DLB; Dementia with Lewy bodies
F; female
FAB; the frontal assessment battery
H/M; the ratio of heart to mediastinum uptake
HRV; heart rate variability
M; male
MIBG; Iodine-123-metaiodobynzylguanidine imaging
MMSE; the mini mental scale examination
SSR; sympathetic skin response
SSR and HRV for Diagnosis of Dementia -1-

Sympathetic Skin Response and Heart Rate Variability as Differential Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study

Masako Negami, MD¹,², Takahiro Maruta, MD, PhD¹,³, Chie Takeda³, Yumi Adachi, MA¹, and Hiroaki Yoshikawa, MD, PhD¹

¹Health Service Center, Kanazawa University
²Health Service Center, Keiju Medical Center
³Neurological Center, Kanazawa-Nishi Hospital

Corresponding author:
Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
SSR and HRV for Diagnosis of Dementia -2-

Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study

Setting: Single centre in Japan

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method.
RESULTS: SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 12.5% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

CONCLUSIONS: These results suggest that SSR and HRV could be useful for differential diagnosis of DLB.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
SSR and HRV for Diagnosis of Dementia -4-

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD). Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, (123)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for DLB. The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained by the written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop (Table). To detect the difference of value of 1 with the desired significance level of 0.05 and the power of 0.80, 20-patients in each group was required. There were no differences in MMSE and FAB scores.
SSR and HRV for Diagnosis of Dementia -5-

between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded the patients who could not show the will of participating in this study.

Testing of autonomic functions

Examinations were performed in quiet room in which patients made themselves relax and comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. The waveforms of SSR appeared 1.5 to 2.5-second after the stimulations. Three waves were recorded for each side (right and left). The filters used in measurement were as follows: High cut; 1KHz, Low cut; 0.1Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves.

The mean of these six amplitudes was used for analysis, and a value under 1.01 mV (mean ± SD of AD patients) was defined as abnormal.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For
recording and analyses of the A-A intervals, an Artett acceleration plethysmography system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method (MEM). The mean of four-two values, 2 from FFT and 2 from MEM, was used. Cut-off value was obtained from ROC curve using DLB as positive level and AD as negative A value. A value under 1.15 (mean–SD of AD cases) was defined as abnormal. The coefficient of variation of A-A intervals (CVAA) was also analyzed. The examinations were performed by a clinical technician with evaluated expertize and the results was reviewed by a special neurologist trained in the electrophysiological laboratory. The technician was blind to the clinical informations of patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curve of the data was drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). The summary of ROC curves in this study was submitted as a supplemental information.
SSR and HRV for Diagnosis of Dementia

Result

There was no adverse events from performing the examinations. Although there was no difference in the data of the CVAA between patients with DLB and AD, the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). The cut-off value for SSR was settled at 0.90 mV and that of HVR was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HVR (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HVR (LF/HF). For detection of DLB, SSR and HVR had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HVR, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HVR, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an alternative examination, we studied the possibility of utilizing SSR and HVR.
SSR and HRV for Diagnosis of Dementia

SSR reflects sympathetic sweat response. Although SSR amplitude was found to be severely reduced in DLB, there have been no reports of comparison of the data of SSR from DLB and AD. In this study, the SSR amplitudes in patients with DLB were smaller than those with AD. HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography. In a previous study, there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination of sympathetic function. From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan. The 3 AD patients with abnormal SSR did not have abnormal MIBG scan. As well, 3 AD patients with abnormal HRV did not have abnormal MIBG scan. Only one AD patient had both abnormal SSR and HRV. We should follow these patients for their clinical manifestations.

From this study, we recommend the utilization of SSR and HRV in routine
SSR and HRV for Diagnosis of Dementia -9-

clinical practice in patients with dementia.

Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253).
References


Figure legends

Figure 1. Autonomic examinations.

A. Records of sympathetic skin response (SSR).

The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of an Alzheimer disease’s (AD) patient. The SSR of DLB showed almost no response after the electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

B. The X-Y plotting of SSR of the patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.90 mV.

C. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.933.

D. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.

There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
### Table. Comparison of DLB with AD

<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10 M and 10 F</td>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>78.7±6.9</td>
<td>78.5±5.0</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>19.2±4.8</td>
<td>19.3±3.6</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
<td>8.5±4.2</td>
<td>8.6±3.9</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD)</td>
<td>1.20±0.16</td>
<td>1.85±0.15</td>
</tr>
<tr>
<td>(range)</td>
<td>(0.95-1.46)</td>
<td>(1.71-2.18)</td>
</tr>
<tr>
<td>SSR (mean ± SD)</td>
<td>0.72 ± 0.82*</td>
<td>2.33 ± 1.32</td>
</tr>
<tr>
<td>(Cut-off: 0.90mV)</td>
<td>(sensitivity: 85.0%)</td>
<td>(specificity: 85.0%)</td>
</tr>
<tr>
<td>HRV (mean ± SD)</td>
<td>0.597 ± 0.524</td>
<td>2.276 ± 1.313</td>
</tr>
<tr>
<td>(Cut-off: 0.933)</td>
<td>(sensitivity: 90.0%)</td>
<td>(specificity: 85.0%)</td>
</tr>
</tbody>
</table>

*p<0.01 compared with DLB and AD
Fisher's exact probability test was used for analysis.

AD: Alzheimer disease
DLB: Dementia with Lewy bodies
F: female
FAB: the frontal assessment battery
H/M: the ratio of heart to mediastinum uptake
HRV: heart rate variability
M: male
MIBG: Iodine-123-metaiodobenzylguanidine imaging
MMSE: the mini mental scale examination
SSR: sympathetic skin response
Figure

A

B

C

D

SSR (mV) vs. DLB and AD

HRV (LF/HF) vs. DLB and AD

CVAA(%) vs. DLB and AD

P<0.01

P<0.01

P>0.05

1 mV

1 second

BMJ Open

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

on November 5, 2021 by guest. Protected by copyright.
STARD checklist for reporting of studies of diagnostic accuracy
(version January 2003)

<table>
<thead>
<tr>
<th>Section and Topic</th>
<th>Item #</th>
<th>On page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE/ABSTRACT/KEYWORDS</td>
<td>1</td>
<td>Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
<td>State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.</td>
</tr>
<tr>
<td>METHODS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>3</td>
<td>The study population: The inclusion and exclusion criteria, setting and locations where data were collected.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?</td>
</tr>
<tr>
<td>Test methods</td>
<td>7</td>
<td>The reference standard and its rationale.</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>The number, training and expertise of the persons executing and reading the index tests and the reference standard.</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>12</td>
<td>Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Methods for calculating test reproducibility, if done.</td>
</tr>
<tr>
<td>RESULTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>14</td>
<td>When study was performed, including beginning and end dates of recruitment.</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).</td>
</tr>
<tr>
<td>Test results</td>
<td>17</td>
<td>Time-interval between the index tests and the reference standard, and any treatment administered in between.</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Any adverse events from performing the index tests or the reference standard.</td>
</tr>
<tr>
<td>Estimates</td>
<td>21</td>
<td>Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>How indeterminate results, missing data and outliers of the index tests were handled.</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Estimates of test reproducibility, if done.</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>25</td>
<td>Discuss the clinical applicability of the study findings.</td>
</tr>
</tbody>
</table>
Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2012-001796.R2</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>24-Nov-2012</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Negami, Masako; Keiju Medical Center, Health Service Center Maruta, Takahiro; Kanazawa-Nishi Hospital, Neurological Center Takeda, Chie; Kanazawa-Nishi Hospital, Neurological Center Adachi, Yumi; Kanazawa University, Health Service Center Yoshikawa, Hiroaki; Kanazawa University, Health Service Center</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Neurology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Diagnostics, Neurology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Dementia &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, CLINICAL PHYSIOLOGY, GERIATRIC MEDICINE, Neurology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study

Masako Negami, MD1,2, Takahiro Maruta, MD, PhD1,3, Chie Takeda3, Yumi Adachi, MA1, and Hiroaki Yoshikawa, MD, PhD1

1Health Service Center, Kanazawa University
2Health Service Center, Keiju Medical Center
3Neurological Center, Kanazawa-Nishi Hospital

Corresponding author:
Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kenazawa-u.ac.jp
Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study

Setting: Single center in Japan

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM).
**Results:** SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 25% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

**Conclusions:** These results suggest that SSR and HRV are shown to have values in differentiating DLB and AD.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
SSR and HRV for Diagnosis of Dementia -4-

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD). Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, (123)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for DLB. The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained by the written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop (Table). To detect the difference of value of 1 with the desired significance level of 0.05 and the power of 0.80, 20-patients in each group was required. There were no differences in MMSE and FAB scores.
between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded the patients who could not show the will of participating in this study.

**Testing of autonomic functions**

Examinations were performed in quiet room in which patients made themselves relax and comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at amplitude of 20 mA with 10-second interval. The waveforms of SSR appeared 1.5 to 2.5-second after the stimulations. Three waves were recorded for each side (right and left). The filters used in measurement were as follows: High cut; 1KHz, Low cut; 0.1Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used for analysis.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For recording and analyses of the A-A intervals, an Artett acceleration plethysmography
system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously.\(^4\) From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM). The mean of two values from MEM was used. Cut-off value was obtained from ROC curve using DLB as positive level and AD as negative. The coefficient of variation of A-A intervals (CVAA) was also analyzed. The examinations were performed by a clinical technician with evaluated expertise and the results was reviewed by a special neurologist trained in the electrophysiological laboratory. The technician was blind to the clinical information of patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curve of the data was drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). The summary of ROC curves in this study was submitted as supplemental information.

Result

There were no adverse events from performing the examinations. Although there was no difference in the data of the CVAA between patients with DLB and AD,
the values of SSR and HVR (LF/ HF) were significantly smaller in patients with DLB than in those with AD (Figure 1A). The cut-off value for SSR was settled at 0.90 mV and that of HRV was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HRV (LF/ HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HRV (LF/ HF). For detection of DLB, SSR and HRV had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HRV, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HRV, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an alternative examination, we studied the possibility of utilizing SSR and HRV.

SSR reflects sympathetic sweat response. SSR amplitude was found severely reduced in DLB. However, there have been no reports of comparison of the data of SSR from DLB and AD. In spite of poor reproducibility of SSR, this study
could show that SSR amplitude in patients with DLB were smaller than those with AD.\textsuperscript{7} HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography.\textsuperscript{4} In a previous study,\textsuperscript{8} there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination of sympathetic function.\textsuperscript{9} From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan. The 3 AD patients with abnormal SSR did not have abnormal MIBG scan. As well, 3 AD patients with abnormal HRV did not have abnormal MIBG scan. Only one AD patient had both abnormal SSR and HRV. We should follow these patients for their clinical manifestations.

Although this study has limitation due to lack of normal control, these results suggest that SSR and HRV have values in differentiating DLB and AD.
Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253).
References


Figure legends

Figure 1. Autonomic examinations.


The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of an Alzheimer disease’s (AD) patient. The SSR of DLB showed almost no response after the electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

2. The X-Y plotting of SSR of the patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.90 mV.

3. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.933.

4. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.

There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
### Table. Comparison of DLB with AD

<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10 M and 10 F</td>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>78.7±6.9</td>
<td>78.5±5.0</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>19.2±4.8</td>
<td>19.3±3.6</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
<td>8.5±4.2</td>
<td>8.6±3.9</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD) (range)</td>
<td>1.20±0.16 (0.95-1.46)</td>
<td>1.85±0.15 (1.71-2.18)</td>
</tr>
<tr>
<td>SSR (mean ± SD)</td>
<td>0.72 ± 0.82* (sensitivity; 85.0%) (specificity; 85.0%)</td>
<td>2.33 ± 1.32</td>
</tr>
<tr>
<td>HRV (mean ± SD)</td>
<td>0.597 ± 0.524 (sensitivity; 90.0%) (specificity; 85.0%)</td>
<td>2.276 ± 1.313</td>
</tr>
</tbody>
</table>

* p<0.01 compared with DLB and AD

Fisher's exact probability test was used for analysis.

AD; Alzheimer disease
DLB; Dementia with Lewy bodies
F; female
FAB; the frontal assessment battery
H/M; the ratio of heart to mediastinum uptake
HRV; heart rate variability
M; male
MIBG; Iodine-123-metaiodobynzylguanidine imaging
MMSE; the mini mental scale examination
SSR; sympathetic skin response
Sympathetic Skin Response and Heart Rate Variability as **Differential Diagnostic Tools for** the **Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study**

Masako Negami, MD\(^1\), Takahiro Maruta, MD, PhD\(^1,3\), Chie Takeda\(^3\), Yumi Adachi, MA\(^1\), and Hiroaki Yoshikawa, MD, PhD\(^1\)

\(^1\)Health Service Center, Kanazawa University
\(^2\)Health Service Center, Keiju Medical Center
\(^3\)Neurological Center, Kanazawa-Nishi Hospital

Corresponding author:

Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
SSR and HRV for Diagnosis of Dementia -2-

Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study

Setting: Single centre in Japan

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method.
Results: SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 25% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

Conclusions: These results suggest that SSR and HRV are shown to have values in differentiating DLB and AD could be useful for differential diagnosis of DLB.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
SSR and HRV for Diagnosis of Dementia -4-

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD). Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, (123)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for DLB. The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained by the written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop (Table). To detect the difference of value of 1 with the desired significance level of 0.05 and the power of 0.80, 20-patients in each group was required. There were no differences in MMSE and FAB scores
SSR and HRV for Diagnosis of Dementia -5-

between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded the patients who could not show the will of participating in this study.

Testing of autonomic functions

Examinations were performed in quiet room in which patients made themselves relax and comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA with 10-second interval. The waveforms of SSR appeared 1.5 to 2.5-second after the stimulations. Three waves were recorded for each side (right and left). The filters used in measurement were as follows: High cut; 1KHz, Low cut; 0.1Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used for analysis and a value under 1.01 mV (mean-SD of AD patients) was defined as abnormal.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes.
recording and analyses of the A-A intervals, an Artett acceleration plethysmography system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the Fast Fourier Transform (FFT) and the maximal entropy method (MEM). The mean of four two values, 2 from FFT and 2 from MEM, was used. Cut-off value was obtained from ROC curve using DLB as positive level and AD as negative A value under 1.15 (mean SD of AD cases) was defined as abnormal. The coefficient of variation of A-A intervals (CVAA) was also analyzed. The examinations were performed by a clinical technician with evaluated expertise and the results was reviewed by a special neurologist trained in the electrophysiological laboratory. The technician was blind to the clinical information of patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curve of the data was drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). The summary of ROC curves in this study was submitted as supplemental information.
Result

There were no adverse events from performing the examinations. Although there was no difference in the data of the CVAA between patients with DLB and AD, the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). The cut-off value for SSR was settled at 0.90 mV and that of HVR was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HRV (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HRV (LF/HF). For detection of DLB, SSR and HRV had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HRV, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HRV, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an alternative examination, we studied the possibility of utilizing SSR and HRV.
SSR and HRV for Diagnosis of Dementia -8-

SSR reflects sympathetic sweat response.\(^6\) SSR amplitude was found severely reduced in DLB. However, there have been no reports of comparison of the data of SSR from DLB and AD. In spite of poor reproducibility of SSR, this study could show that SSR amplitude in patients with DLB were smaller than those with AD.\(^7\). Although SSR amplitude was found to be severely reduced in DLB, there have been no reports of comparison of the data of SSR from DLB and AD.\(^7\). In this study, the SSR amplitudes in patients with DLB were smaller than those with AD.\(^7\). HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography.\(^4\) In a previous study,\(^8\) there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination of sympathetic function.\(^9\) From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan.\(^7\) The 3 AD patients with abnormal SSR did not have abnormal MIBG scan.\(^7\). As well, 3 AD patients with abnormal HRV did not have abnormal MIBG scan.
Only one AD patient had both abnormal SSR and HRV. We should follow these patients for their clinical manifestations.

Although this study has limitation due to lack of normal control, these results suggest that SSR and HRV have values in differentiating DLB and AD. From this study, we recommend the utilization of SSR and HRV in routine clinical practice in patients with dementia.

Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253).
SSR and HRV for Diagnosis of Dementia -10-

References


Figure legends

Figure 1. Autonomic examinations.
   The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of an Alzheimer disease’s (AD) patient. The SSR of DLB showed almost no response after the electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

2. The X-Y plotting of SSR of the patients with DLB or AD.
   The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.90 mV.

3. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.
   The ROC curve was drawn using the data of DLB and AD. Accordingly, cut-off value was settled at 0.933.

4. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.
   There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
SSR and HRV for Diagnosis of Dementia

<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10 M and 10 F</td>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>78.7±6.9</td>
<td>78.5±5.0</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>19.2±4.8</td>
<td>19.3±3.6</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
<td>8.5±4.2</td>
<td>8.6±3.9</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD)</td>
<td>1.20±0.16 (range 0.95-1.46)</td>
<td>1.85±0.15 (range 1.71-2.18)</td>
</tr>
<tr>
<td>SSR (mean ± SD)</td>
<td>0.72 ± 0.82* (Cut-off: 0.90mV) (sensitivity: 85.0%) (specificity: 85.0%)</td>
<td>2.33 ± 1.32</td>
</tr>
<tr>
<td>HRV (mean ± SD)</td>
<td>0.597 ± 0.524 (Cut-off: 0.933) (sensitivity: 90.0%) (specificity: 85.0%)</td>
<td>2.276 ± 1.313</td>
</tr>
</tbody>
</table>

*p<0.01 compared with DLB and AD

Fisher's exact probability test was used for analysis.

AD: Alzheimer disease
DLB: Dementia with Lewy bodies
F: female
FAB: the frontal assessment battery
H/M: the ratio of heart to mediastinum uptake
HRV: heart rate variability
M: male
MIBG: Iodine-123-metaiodobenzylguanidine imaging
MMSE: the mini mental scale examination
SSR: sympathetic skin response
Figure

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml
# STARD checklist for reporting of studies of diagnostic accuracy (version January 2003)

<table>
<thead>
<tr>
<th>Section and Topic</th>
<th>Item #</th>
<th>On page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE/ABSTRACT/KEYWORDS</td>
<td>1</td>
<td>Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
<td>State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.</td>
</tr>
<tr>
<td>METHODS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>3</td>
<td>The study population: The inclusion and exclusion criteria, setting and locations where data were collected.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?</td>
</tr>
<tr>
<td>Test methods</td>
<td>7</td>
<td>The reference standard and its rationale.</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>The number, training and expertise of the persons executing and reading the index tests and the reference standard.</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>12</td>
<td>Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Methods for calculating test reproducibility, if done.</td>
</tr>
<tr>
<td>RESULTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants</td>
<td>14</td>
<td>When study was performed, including beginning and end dates of recruitment.</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).</td>
</tr>
<tr>
<td>Test results</td>
<td>17</td>
<td>Time-interval between the index tests and the reference standard, and any treatment administered in between.</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Any adverse events from performing the index tests or the reference standard.</td>
</tr>
<tr>
<td>Estimates</td>
<td>21</td>
<td>Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>How indeterminate results, missing data and outliers of the index tests were handled.</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Estimates of test reproducibility, if done.</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>25</td>
<td>Discuss the clinical applicability of the study findings.</td>
</tr>
</tbody>
</table>
Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study

<table>
<thead>
<tr>
<th>Journal:</th>
<th>BMJ Open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manuscript ID:</td>
<td>bmjopen-2012-001796.R3</td>
</tr>
<tr>
<td>Article Type:</td>
<td>Research</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>18-Jan-2013</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
<td>Negami, Masako; Keiju Medical Center, Health Service Center Maruta, Takahiro; Kanazawa-Nishi Hospital, Neurological Center Takeda, Chie; Kanazawa-Nishi Hospital, Neurological Center Adachi, Yumi; Kanazawa University, Health Service Center Yoshikawa, Hiroaki; Kanazawa University, Health Service Center</td>
</tr>
<tr>
<td>Primary Subject Heading:</td>
<td>Neurology</td>
</tr>
<tr>
<td>Secondary Subject Heading:</td>
<td>Diagnostics, Neurology</td>
</tr>
<tr>
<td>Keywords:</td>
<td>Dementia &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, Neurophysiology &lt; NEUROLOGY, CLINICAL PHYSIOLOGY, GERIATRIC MEDICINE, Neurology &lt; INTERNAL MEDICINE</td>
</tr>
</tbody>
</table>
Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study

Masako Negami, MD\textsuperscript{1,2}, Takahiro Maruta, MD, PhD\textsuperscript{1,3}, Chie Takeda\textsuperscript{3}, Yumi Adachi, MA\textsuperscript{1}, and Hiroaki Yoshikawa, MD, PhD\textsuperscript{1,4}

\textsuperscript{1}Health Service Center, Kanazawa University

\textsuperscript{2}Health Service Center, Keiju Medical Center

\textsuperscript{3}Neurological Center, Kanazawa-Nishi Hospital

\textsuperscript{4}Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science

Corresponding author:

Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic skin response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study.

Setting: Single center in Japan.

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM).
**Results:** SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 25% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

**Conclusions:** SSR and HRV can be applied to differentiate DLB from AD.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD).\(^1\) Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, \(^{123}\)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnosis of DLB.\(^2\) MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of MIBG scan.

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained using a written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria\(^3\) and 20 with probable DLB diagnosed with the criteria of the 3\(^{rd}\) international DLB workshop\(^2\) (Table). To detect a difference of a value of 1 at the desired significance level of 0.05 and power of 0.80, 20 patients in each group were required. There were no differences in MMSE and FAB scores
between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded patients not willing to participate in the study.

Testing of autonomic functions

Examinations were performed in a quiet room in which patients relaxed and made themselves comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA with a 10-second interval. The waveforms of SSR appeared 1.5 to 2.5 seconds after the stimulations. Three waves were recorded for each side (right and left). The filters used in the measurement were as follows: high cut, 1 KHz; low cut, 0.1 Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used for analysis.

For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For recording and analyses of the A-A intervals, an Artett acceleration plethysmography
system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM). The mean of two values from MEM was used. Cut-off value was obtained from an ROC curve using DLB as positive level and AD as negative. The coefficient of variation of A-A intervals (CVAA) was also analyzed. The examinations were performed by a clinical technician with extensive experience of such work and the results were reviewed by a special neurologist trained for working in an electrophysiological laboratory. The technician was blind to the clinical information of the patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curves of the data were drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). A summary of the ROC curves in this study is available as supplemental information.

Result

There were no adverse events resulting from performing the examinations.
Although there was no difference in the data of the CVAA between patients with DLB and AD, the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). The cut-off value for SSR was set at 0.90 mV and that of HRV was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HRV (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HRV (LF/HF). For detection of DLB, SSR and HRV had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HRV, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HRV, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. MIBG scan is a useful examination for the detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an alternative examination, we studied the possibility of utilizing SSR and HRV.

SSR reflects sympathetic sweat response. SSR amplitude was found to be severely reduced in DLB. However, there are no reports of a comparison of the data of...
SSR between DLB and AD. In spite of the poor reproducibility of SSR, this study could show that SSR amplitude in patients with DLB was smaller than in those with AD.\textsuperscript{7} HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography.\textsuperscript{4} In a previous study,\textsuperscript{8} there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as to examine sympathetic function.\textsuperscript{9} From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as a reflection of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan. The 3 AD patients with abnormal SSR did not have abnormal MIBG scan. In addition, 3 AD patients with abnormal HRV did not have abnormal MIBG scan. Only one AD patient had abnormalities of both SSR and HRV. We should follow these patients for their clinical manifestations.

Although this study has a limitation due to its lack of a normal control, it shows that SSR and HRV can be applied to differentiate DLB from AD.
Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253). English language in this manuscript is edited by Medical English Service (Kyoto, Japan).
References


SSR and HRV for Diagnosis of Dementia -11-

Figure legends

Figure 1. Autonomic examinations.


   The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of a patient with Alzheimer’s disease (AD). The SSR of DLB showed almost no response after electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude larger than 1 mV).

2. The X-Y plotting of SSR of the patients with DLB or AD.

   The ROC curve was drawn using the data of DLB and AD. Accordingly, a cut-off value was set at 0.90 mV.

3. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.

   The ROC curve was drawn using the data of DLB and AD. Accordingly, a cut-off value was set at 0.933.

4. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.

   There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
Table. Comparison of DLB with AD

<table>
<thead>
<tr>
<th></th>
<th>DLB (n=20)</th>
<th>AD (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>10 M and 10 F</td>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
<td>78.7±6.9</td>
<td>78.5±5.0</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>19.2±4.8</td>
<td>19.3±3.6</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
<td>8.5±4.2</td>
<td>8.6±3.9</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD) (range)</td>
<td>1.20±0.16 (0.95-1.46)</td>
<td>1.85±0.15 (1.71-2.18)</td>
</tr>
<tr>
<td>SSR (mean ± SD)</td>
<td>0.72 ± 0.82* (sensitivity: 85.0%) (specificity: 85.0%)</td>
<td>2.33 ± 1.32</td>
</tr>
<tr>
<td>SSR (cut-off: 0.90 mV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRV (mean ± SD)</td>
<td>0.597 ± 0.524 (sensitivity: 90.0%) (specificity: 85.0%)</td>
<td>2.276 ± 1.313</td>
</tr>
<tr>
<td>HRV (cut-off: 0.933)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* p<0.01, comparing DLB with AD

Fisher's exact probability test was used for analysis.

AD: Alzheimer's disease
DLB: dementia with Lewy bodies
F: female
FAB: the frontal assessment battery
H/M: the ratio of heart to mediastinum uptake
HRV: heart rate variability
M: male
MIBG: 123I-iodine-metaiodobenzylguanidine imaging
MMSE: Mini Mental State Examination
SSR: sympathetic skin response
SSR and HRV for Diagnosis of Dementia -1-

Sympathetic Skin Response and Heart Rate Variability as Diagnostic Tools for the Differential Diagnosis of Lewy Body Dementia and Alzheimer’s Disease: A Diagnostic Test Study

Masako Negami, MD1,2, Takahiro Maruta, MD, PhD1,3, Chie Takeda3, Yumi Adachi, MA1, and Hiroaki Yoshikawa, MD, PhD1,4

1Health Service Center, Kanazawa University
2Health Service Center, Keiju Medical Center
3Neurological Center, Kanazawa-Nishi Hospital
4Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science

Corresponding author:–

Hiroaki Yoshikawa, Health Service Center, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan, Tel.: +81-76-264-5254, Fax: +81-76-234-4044, E-mail: hiroaki@staff.kanazawa-u.ac.jp
SSR and HRV for Diagnosis of Dementia -2-

Abstract

Objective: The purpose of this study is to investigate the usefulness of sympathetic response (SSR) and heart rate variability (HRV) for the differential diagnosis of patients with dementia with Lewy bodies (DLB).

Design: Diagnostic test study.

Setting: Single center in Japan.

Participants: We examined 20 patients with probable Alzheimer’s disease (AD) diagnosed with NINCDS-ADRDA criteria and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop.

Methods: For the SSR measurement, surface electrodes were used: the active electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA. For the HRV measurement, the A-A intervals were measured twice for two minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM).
SSR and HRV for Diagnosis of Dementia -3-

Results: SSR and HRV could detect abnormality of autonomic function in patients with DLB at sensitivities of 85.0% and 90.0%, respectively. On the other hand, SSR and HRV detected abnormality of autonomic function in patients with AD at sensitivities of 15.0% and 25% (p<0.05). The combination of SSR and HRV (double-positive) indicated abnormal autonomic function in only 1 out of 20 patients (5%) with AD. In contrast, this combination indicated autonomic abnormality in 15 out of 20 patients with DLB by our criteria (75%).

Conclusions: SSR and HRV can be applied to differentiate DLB from AD.

Key words: Dementia with Lewy bodies, Alzheimer’s disease, Sympathetic skin response, Heart rate variability
SSR and HRV for Diagnosis of Dementia -4-

Introduction

Dementia with Lewy bodies (DLB) is the second most common cause of degenerative dementia after Alzheimer’s disease (AD).\(^1\) Because of the difficulty of distinguishing DLB from AD owing to overlapping clinical features, \(^{123}\)I-metaiodobenzylguanidine (MIBG) scan is described as a supportive examination in the diagnostic criteria for diagnosis of DLB.\(^2\) The MIBG scan is regarded as a useful examination of sympathetic function. However, the utilization of a radioisotope (RI), high running costs and long testing time prevent the MIBG scan from becoming a routine clinical examination. Here, we investigate the utilization of other autonomic examinations, that is, sympathetic skin response (SSR) and heart rate variability (HRV), instead of the MIBG scan.\(^-\)

Patients and methods

Patients

This study was approved by the ethics committee of Kanazawa-Nishi Hospital. The patients’ consent was obtained by the using a written consent form. The test was performed from 2009 to 2010. We tested 20 Japanese patients with probable AD diagnosed with NINCDS-ADRDA criteria\(^3\) and 20 with probable DLB diagnosed with the criteria of the 3rd international DLB workshop\(^2\) (Table). To detect the difference of a value of 1 without the desired significance level of 0.05 and the power of 0.80, 20 patients in each group were required. There were no differences in MMSE and...
SSR and HRV for Diagnosis of Dementia -5-

FAB scores between the DLB group and the AD group. We evaluated the ratio of heart to mediastinum uptake (H/M) of the MIBG scan using single-photon emission computed tomography (SPECT). The values of H/M ratio in all AD patients were larger than 1.70 and those of all DLB patients were smaller than 1.50. We excluded patients with cardiovascular disease including arrhythmia, diabetes mellitus, other degenerative diseases and peripheral neuropathies. We also excluded the patients who could not show the will of participating in this study.

Testing of autonomic functions

Examinations were performed in a quiet room in which patients relaxed and made themselves relax and comfortable. We also offered patients a rest after examinations.

For the SSR measurement, surface electrodes were used: the active recording electrode was placed on the palm of the hand and the reference electrode was placed on the dorsum of the same hand. SSR was induced by median nerve electrical stimulation at an amplitude of 20 mA with a 10-second interval. The waveforms of SSR appeared 1.5 to 2.5 seconds after the stimulations. Three waves were recorded for each side (right and left). The filters used in the measurement were as follows: high cut: 1 KHz, low cut: 0.1 Hz. The measured amplitude was defined as the peak-to-peak value of the recorded waves. The mean of these six amplitudes was used for analysis.

For the HRV measurement, the A-A intervals were measured twice for two
For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

SSR and HRV for Diagnosis of Dementia -6-

minutes with an interval of 5 minutes in a sitting position after a rest of 5 minutes. For recording and analyses of the A-A intervals, an Artett acceleration plethysmography system (U-Medica, Osaka, Japan) was utilized for analyses of the data, as described previously. 4 From the low-frequency power (LF; 0.02-0.15 Hz) and the high-frequency power (HF; 0.15-0.50 Hz), the ratio of LF to HF power (LF/HF) was calculated using the maximal entropy method (MEM). The mean of two values from MEM was used. Cut-off value was obtained from an ROC curve using DLB as positive level and AD as negative. The coefficient of variation of A-A intervals (CVAA) was also analyzed. The examinations were performed by a clinical technician with evaluated experience of such work and the results were reviewed by a special neurologist trained for working in the electrophysiological laboratory. The technician was blind to the clinical information of the patients.

For statistical analyses, the data were first tested for a normal distribution using the Shapiro-Wilk test. In the categories with a normal distribution, data were analyzed for equality of variance by F-test, and then Student’s t-test or Welch’s t-test was utilized. In the categories with a non-normal distribution, Wilcoxon’s test was utilized. To detect abnormality in the SSR or HRV examination, Fisher’s exact test was used. The SSR and HRV examinations were performed in a quiet room and the patients were kept awake and relaxed during the procedures. The ROC curves of the data were drawn using JMP 10.0.1 (SAS Institute Inc., Cary, NC, USA). The summary of the ROC curves in this study was submitted as supplemental information.
SSR and HRV for Diagnosis of Dementia -7-

Result

There were no adverse events resulting from performing the examinations. Although there was no difference in the data of the CVAA between patients with DLB and AD, the values of SSR and HVR (LF/HF) were significantly smaller in patients with DLB than in those with AD (Figure 1 A). The cut-off value for SSR was settled at 0.90 mV and that of HRV was 0.933. Regarding the 20 patients with DLB, 17 were classified as abnormal for the SSR and 18 were classified as abnormal for the HRV (LF/HF). Regarding the 20 patients with AD, 3 were classified as abnormal for the SSR and 5 were classified as abnormal for the HRV (LF/HF). For detection of DLB, SSR and HRV had sensitivities of 85.0% and 90.0% and specificities of 85.0% and 85.0%, respectively. While 15 out of 20 patients with DLB (75.0%) showed double-abnormality in SSR and HRV, only 1 out of 20 patients with AD (5.0%) had double-abnormality status. While 20 of 20 cases with DLB (100.0%) were abnormal in either SSR or HRV, 7 of 20 patients with AD (35.0%) were abnormal in either SSR or HRV (Table).

Discussion

Autonomic dysfunction often appears in patients with DLB. The MIBG scan is a useful examination for the detection of sympathetic activity and is utilized to distinguish DLB from AD. Since MIBG scans require an RI and a long testing period (more than 3 hours), they are not suitable in a routine clinical setting. As an
alternative examination, we studied the possibility of utilizing SSR and HRV.

SSR reflects sympathetic sweat response. SSR amplitude was found to be severely reduced in DLB. However, there have been no reports of a comparison of the data of SSR between DLB and AD. In spite of the poor reproducibility of SSR, this study could show that SSR amplitude in patients with DLB was smaller than in those with AD. HRV reflects autonomic heart rate response and is detectable by acceleration plethysmography. In a previous study, there was no significant difference of single LF and single HF between patients with DLB and those with AD. Therefore, we investigated the ratio of LF to HF (LF/HF) instead. LF/HF is usually used as an examination to examine sympathetic function. From our results, patients with DLB showed lower values of LF/HF than those with AD. We also analyzed the CVAA as an examination of parasympathetic activity and found no difference between patients with AD and those with DLB. These results suggest that the sympathetic systems are impaired in DLB.

We also investigated the diagnostic accuracy of combination analyses using SSR and HRV. We found 16 patients with a double-abnormal result. The MIBG scan classified them as follows: 15 with DLB and 1 with AD. All 20 patients diagnosed by an MIBG scan as having DLB could be correctly classified using either SSR or HRV. Thus, the combined analysis using SSR and HRV is useful and could be a substitute for the MIBG scan. The 3 AD patients with abnormal SSR did not have abnormal MIBG scan. As well, 3 AD patients with abnormal HRV did not have abnormal MIBG scan. Only one AD patient had abnormalities of both abnormal SSR and HRV.
We should follow these patients for their clinical manifestations.

Although this study has a limitation due to its lack of a normal control, it shows that SSR and HRV have their applicability can be applied to differentiate DLB from AD.²

Acknowledgments

This work was supported in part by a Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan and by KAKENHI (24591253). English language in this manuscript is edited by Medical English Service (Kyoto, Japan).
References


SSR and HRV for Diagnosis of Dementia

Figure legends

Figure 1. Autonomic examinations.


The left scheme is a typical record of a patient with dementia with Lewy bodies (DLB) and the right scheme is that of a patient with Alzheimer’s disease (AD). The SSR of DLB showed almost no response after electric stimulation, while that of AD showed a remarkable response (peak-to-peak amplitude is larger than 1 mV).

2. The X-Y plotting of SSR of the patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, a cut-off value was settled at 0.90 mV.

3. The X-Y plotting of heart rate variability (HRV) of patients with DLB or AD.

The ROC curve was drawn using the data of DLB and AD. Accordingly, a cut-off value was settled at 0.933.

4. The X-Y plotting of the coefficient of variation of A-A interval (CVAA) of patients with DLB or AD.

There was no difference between the values of CVAA of patients with AD (2.91±2.36) and of those with DLB (3.73±3.44).
<table>
<thead>
<tr>
<th>Table. Comparison of DLB with AD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td><strong>DLB</strong></td>
</tr>
<tr>
<td>(n=20)</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>10 M and 10 F</td>
</tr>
<tr>
<td>Age (mean ±SD)</td>
</tr>
<tr>
<td>78.7±6.9</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
</tr>
<tr>
<td>19.2±4.8</td>
</tr>
<tr>
<td>FAB (mean ± SD)</td>
</tr>
<tr>
<td>8.5±4.2</td>
</tr>
<tr>
<td>H/M of MIBG (mean ± SD)</td>
</tr>
<tr>
<td>(range)</td>
</tr>
<tr>
<td>1.20±0.16 (0.95-1.46)</td>
</tr>
<tr>
<td>SSR (mean ± SD)</td>
</tr>
<tr>
<td>0.72 ± 0.82* (Cut-off 0.90mV)</td>
</tr>
<tr>
<td>(specificity 85.0%)</td>
</tr>
<tr>
<td>HRV (mean ± SD)</td>
</tr>
<tr>
<td>0.597 ± 0.524 (Cut-off 0.933)</td>
</tr>
<tr>
<td>(specificity 85.0%)</td>
</tr>
</tbody>
</table>

*p < 0.01 comparing DLB with DLB and AD.

Fisher’s exact probability test was used for analysis.

AD: Alzheimer’s disease
DLB: Dementia with Lewy bodies
F: female
FAB: the Frontal Assessment Battery
H/M: the ratio of heart to mediastinum uptake
HRV: heart rate variability
M: male
MIBG: iodine-123-metaiodobenzylguanidine imaging
MMSE: mini mental state examination
SSR: sympathetic skin response
Figure A

B

C

D

SSR (mV)

P<0.01

P<0.01

P>0.05

DLPB

AD

HRV (LF/HF)

DLPB

AD

CVA (%)

DLPB

AD

508x381mm (72 x 72 DPI)
**Receiver Operating Characteristic**

- **Fit Model DLB-AD SSR**
  - AUC: 0.88125
- **Fit Model DLB-AD LF/HF(MEM)**
  - AUC: 0.91875
- **Fit Model DLB-AD CVAA**
  - AUC: 0.50500
- **Fit Model DLB-Norm LF/HF(MEM)**
  - AUC: 0.82250

- **Fit Model DLB-Norm CVAA**
  - AUC: 0.36000

Using Diag="DLB" to be the positive level.
# STARD checklist for reporting of studies of diagnostic accuracy  
*(version January 2003)*

<table>
<thead>
<tr>
<th>Section and Topic</th>
<th>Item #</th>
<th>Item</th>
<th>On page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE/ABSTRACT/KEYWORDS</td>
<td>1</td>
<td>Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').</td>
<td>1, 2, 3, 4</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>2</td>
<td>State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.</td>
<td>5</td>
</tr>
<tr>
<td>METHODS</td>
<td>3</td>
<td>The study population: The inclusion and exclusion criteria, setting and locations where data were collected.</td>
<td>4, 5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?</td>
<td>4, 5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.</td>
<td>4, 5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?</td>
<td>4, 5</td>
</tr>
<tr>
<td>Test methods</td>
<td>7</td>
<td>The reference standard and its rationale.</td>
<td>5, 6</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.</td>
<td>5, 6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.</td>
<td>4, 5, 6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>The number, training and expertise of the persons executing and reading the index tests and the reference standard.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.</td>
<td>6</td>
</tr>
<tr>
<td>Statistical methods</td>
<td>12</td>
<td>Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>Methods for calculating test reproducibility, if done.</td>
<td>N/A</td>
</tr>
<tr>
<td>RESULTS</td>
<td>14</td>
<td>When study was performed, including beginning and end dates of recruitment.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).</td>
<td>4</td>
</tr>
<tr>
<td>Test results</td>
<td>17</td>
<td>Time-interval between the index tests and the reference standard, and any treatment administered in between.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.</td>
<td>The data was sent to Dryad</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Any adverse events from performing the index tests or the reference standard.</td>
<td>6</td>
</tr>
<tr>
<td>Estimates</td>
<td>21</td>
<td>Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>How indeterminate results, missing data and outliers of the index tests were handled.</td>
<td>4, 5, 6</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Estimates of test reproducibility, if done.</td>
<td>NA</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>25</td>
<td>Discuss the clinical applicability of the study findings.</td>
<td>8</td>
</tr>
</tbody>
</table>