Video Pupillometric Evaluation of the Pupillary Reflex as a Test for the Clinical Manifestations of Parkinson’s Disease

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Abstract

Purpose. The aim was to investigate the utility of the eye-tracking pupillary reflex when testing for autonomic dysfunction in Parkinson’s disease (PD) patients by correlating the results with clinical status.

Methods. The eye-tracking video pupillometric evaluation of the pupillary reflex to light and the isometric hand grip task were measured in 45 PD patients (Hoehn & Yahr stage, 2-4; mean age, 72.7 ± 9.9 years; disease duration 7.2 ± 5.0 years; male, 53.3%). We performed consecutive measurements of pupil size, which were expressed as the number of pixels, and measured the light miosis response (LMiR) and light mydriasis response (LMyR) to changes in luminance (80 Lux and 400 Lux). We also calculated the mydriasis response to the isometric hand grip task (HMyR) as ratios to the pupil size before the stimulus.

Results. LMiR and LMyR were significantly smaller in PD patients than in controls (p = 0.002 and p = 0.006, respectively). Pupil size before and after the hand grip task and HMyR were similar to normal control values. LMiR in PD patients significantly correlated with Movement Disorder Society- Unified Parkinson’s Disease Rating Scale (MDS-UPDRS) part 3 (p = 0.011) and part 4 (p = 0.003). LMyR correlated with MDS-UPDRS part 4 (p = 0.016). HMyR correlated with disease duration (p = 0.007) and levodopa equivalent daily dose (p = 0.025). Multiple linear regression analyses demonstrated that MDS-UPDRS part 3 (p = 0.005) was significantly associated with LMiR. Disease duration (p = 0.037) was significantly associated with LMyR. But there was no clinical factor associated with HMyR.

Conclusion. An abnormal pupillary reflex, such as a low response to light stimulation and a high response to the hand grip task, may be observed in the advanced stage of PD.

Key words

Parkinson’s disease, pupillary reflex, video pupillometric evaluation

Introduction

The pathological process leading to Parkinson’s disease (PD) begins decades before the characteristic motor symptoms. This premotor phase of PD may include dysautonomia, which typically exhibits subtle signs and symptoms, and has not been fully explained. Although there are few options for the quantitative evaluation methods of dysautonomia, 123I-metaiodobenzylguanidine (MIBG) cardiac scintigraphy has been established as a validated method of evaluating postganglionic presynaptic cardiac sympathetic innervation and is used to diagnose PD. Cardiac denervation in PD patients correlates strongly with early cardiac MIBG uptake, and MIBG cardiac scintigraphy has been used to differentiate PD from other neurodegenerative disorders. Thus, abnormal MIBG myocardial scintigraphy was adopted as supportive evidence in the recently published diagnostic criteria for PD in 2015 by the Movement Disorder Society.

Recent advances in eye-tracking technology enable pupill size measurement at various temporal rates. Pupil dilation is modulated by the activity of...
the locus coeruleus in the noradrenergic system, and such pupillometry may offer new insights into dysautonomia in PD. The aim of this study was to investigate the utility of eye-tracking video pupillometric measurements of the pupillary reflex to light stimulation and the isometric hand grip task in PD patients by correlating the results with clinical status and abnormalities on MIBG myocardial scintigraphy.

Materials and Methods

Study Design and Registration.

This study was conducted in a single center, and the study protocol was approved by the St. Marianna University Bioethics Committee (approval no. 2710). All participants, including normal control subjects provided written, informed consent before the study started.

Study population.

A total of 45 patients with PD who met the following inclusion criteria were prospectively enrolled between August 2017 and October 2018: 1) patients who fulfilled the diagnostic criteria described by the UK Parkinson Disease Society brain bank; 2) patients aged ≥20 years and <90 years; 3) patients with a Hoehn-Yahr (H-Y) Stage between II and IV; and 4) patients with the ability to comply with the study protocol. The following patients were excluded: 1) patients who had undergone deep brain stimulation surgery; 2) patients suffering from ophthalmological diseases such as cataracts, glaucoma, or retinal diseases; 3) patients with a history of intraocular surgery; 4) patients suffering from hallucinations or delusions; 5) patients with moderate dementia (the Revised Hasegawa Dementia Scale (HDS-R) <16); or 6) patients with a history of changes in medication for PD within two months. No restrictions were placed on disease duration. In this study, 20 normal control subjects (mean age, 45.5 ± 16.6 years; male, 45%) were also enrolled to obtain normal values for PD within two months. No restrictions were placed on disease duration. In this study, 20 normal control subjects (mean age, 45.5 ± 16.6 years; male, 45%) were also enrolled to obtain normal values for MIBG myocardial scintigraphy.

Eye-tracking video pupillometric evaluation.

All experimental sessions were conducted between 12:00 and 18:00 to control for the effects of circadian rhythm. Laboratory illumination and temperature were maintained at 60 – 100 lux and 23 – 26°C, respectively. We measured pupillary reflexes in response to two stimuli: light and an isometric hand grip task. The presence of miosis and mydriasis in response to changes in the level of ambient light are measured to represent the pupillary reflex and primarily reflect the function of the light reflex pathway in the midbrain. Conversely, pupillary measurements during an isometric hand grip task are believed to primarily reflect the mydriatic response to activation of the sympathetic nervous system initiated by a powerful hand grip. The hand grip test is used to evaluate psychological stress, and may indicate damage to the sympathetic pathways over a more extensive anatomical area than the midbrain.

On arrival at the laboratory, subjects sat still for 30 min, then we measured their pupillary reflexes in response to light. The subjects then performed the isometric hand grip task. To measure the pupillary light reflex, we measured pupil size in a resting state (illumination, 80 lux) for 10 s, during light stimulation (400 lux) for 10 s, and again as the light stimulation was being removed (from 400 to 80 lux). Subjects performed isometric hand grip tasks using a medium-resistance hand therapy exercise ball (Gaiam) according to the methods described by Nielsen et al. Subjects chose the rubber ball that was easiest to grip from among three different types. We measured pupil size in a resting state for 10 s before task onset and then for the next 18 s while subjects gripped the ball as hard as possible. The changes in pupil size were recorded over the entire 28 seconds, and we reported each measurement three times.

We performed the pupillary reflex measurements using the ETL-400 eye-tracking system (ISCAN Inc., MA, USA) at a sampling rate of 60 Hz. This system is configured to image and track a single eye at a time. The corneal reflection is generated by the reflection of the infrared light source used to illuminate the eye area and track both the X, Y position of the subject’s dark pupil and the X, Y position of the bright corneal reflection. The display screen for the real-time video eye monitoring used to track the eye is represented by a 512-pixel-horizontal by a 256-pixel-vertical matrix. Representative recordings are shown in Figure 1 and 2.

Mean resting pupil size was calculated based on a total of 600 samples of pupil area measured for 10 s before stimulation. Mean pupil sizes during stimulation were calculated for the 20 largest pupil size sam-
Figure 1. Eye tracking pupillometric evaluation of the pupillary responses to light and the isometric hand grip task recorded in a 43-year-old healthy woman.
1. Participants rest for 30 minutes under 80–100 lux lighting.
2. Participants are seated at 30 cm from the CCD camera for the eye tracking system and focus on it. <Start>
3. Rest for 10 seconds (Patients are being monitored during this time).
4. Perform each test (Hand grip task, Light stimulus).

Figure 2. Sequential changes in pupil size in an 82-year-old Parkinson’s disease patient.
In this PD patient, the change in pupil diameter from light stimulation to after light stimulation removal was small, while mydriasis increased during hand grip stimulation.

To evaluate the pupillary reflex response to the changes in luminance and to the isometric hand grip task, we measured pupil size as the number of pixels. The light miosis response (LMIr) and light mydriasis response (LMyr) to the changes in luminance and the mydriasis response to the hand grip task (HMyr) were calculated as follows:

- LMIr (%) = ([Average of minimum 20 pupil sizes during 10 seconds of light stimulus – Average resting state pupil size]/[ Average resting state pupil size]×100)  
- LMyr (%) = ([Average of top 20 pupil sizes after 10 seconds of light stimulus – Average...
of minimum 20 pupil sizes during 10 seconds of light stimulus]/{Average of minimum 20 pupil sizes during 10 seconds of light stimulus}×100)

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\text{HM} \text{yR} \% = ([\text{Average of top 20 pupil sizes during 18 seconds of hand grip task} – \text{Average of Average of resting state pupil size}] / \text{[Average of resting state pupil size]} × 100)
\]

After reviewing all three results, we performed the analysis using the result with the maximum rate of change.

**Statistical Analyses.**

Our statistical analysis was performed using IBM SPSS Statistics ver. 25 (IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY). The Kolmogorov-Smirnov test was performed as a normality test. Pupillary response rates showed a non-normal distribution, and log transformation was applied to transform the distribution of these data, after which the data followed a normal distribution. The log-transformed data and other data with a normal distribution are expressed as means and standard deviations, and we compared the data using Student’s t-test. Spearman’s correlation coefficient was used for the correlation analyses. We also performed multiple linear regression analysis to identify clinical factors associated with LMiR, LMyR, and HMyR. A p value of <0.05 was considered significant (2-tailed).

**Results**

**Characteristics and pupillary responses to each stimulus in patients with Parkinson’s disease and control subjects.**

Table 1 shows the characteristics of the 45 PD patients (mean age, 72.7 ± 9.9 years; male, 53.3%). Comparisons of LMiR, LMyR, and HMyR normalized by logarithmic transformation between the control and PD groups showed significantly lower LMiR (p = 0.002) and LMyR (p = 0.006) in the PD group. No significant differences in pupil diameter were observed between the control and PD groups before light stimulation, immediately after light stimulation being applied, or after light stimulation removal. No

| Table 1. Characteristics of Patients with Parkinson’s Disease and Control Group. |
|---------------------------------------------|-----------------|-----------------|
|                                           | Parkinson’s disease | Control subjects |
|                                           | (n = 45)          | (n = 20)        |
| Age, (years)                              | 72.7 ± 9.9        | 45.5 ± 16.6     |
| Male, n (%)                               | 24 (53.3)         | 9 (45)          |
| Hypertension, n (%)                       | 15 (33.3)         | 2 (10)          |
| Diabetes, n (%)                           | 7 (15.6)          | 0 (0)           |
| Orthostatic hypotension, n (%)            | 9 (20)            | 0 (0)           |
| Hoehn & Yahr Stage, n (%)                 |                  |                 |
| Stage 2                                   | 14 (31.1)         |                 |
| Stage 3                                   | 21 (46.7)         |                 |
| Stage 4                                   | 10 (22.2)         |                 |
| LEDD, mg/day                              | 644.3 ± 369.3     |                 |
| MDS-UPDRS Total                           | 63.0 ± 33.0       |                 |
| Part1                                      | 12.4 ± 8.6        |                 |
| Part2                                      | 17.0 ± 10.6       |                 |
| Part3                                      | 28.9 ± 15.2       |                 |
| Part4                                      | 4.7 ± 5.1         |                 |

MDS-UPDRS: Movement Disorder Society-sponsored revision of the Unified Parkinson’s Disease Rating Scale; LEDD: levodopa equivalent daily dose.

Values are means ± standard deviation.
significant differences in pupil diameter or HMyR were observed between the two groups before or after isometric hand grip task onset (Table 2). Representative case was shown in Figure 3.

**Correlation coefficients between pupillary response and clinical variables.**

Correlations between clinical variables and LMiR, LMyR, and HMyR in the PD group are shown in Table 3. Significant positive correlations were observed for LMiR with MDS-UPDRS part 3 (r = 0.389, p = 0.011) and 4 (r = 0.454, p = 0.003). A negative correlation was observed between LMyR and MDS-UPDRS part 4 (r = -0.371, p = 0.016), whereas significant positive correlations were observed for HMyR with disease duration (r = 0.394, p = 0.007) and LEDD (r = 0.333, p = 0.025). A significant correlation was observed between LMyR and LMiR (r = -0.788, p = 0.001); however, no correlations were observed for HMyR with LMiR or LMyR.

**Multiple linear regression analyses.**

Multiple linear regression analysis was performed for each pupillary response rate (Table 4). No significant factors were observed for HMyR. MDS-UPDRS part3 (p = 0.005) and for LMyR, disease duration (p = 0.037) were identified as significant factors for LMiR (Table 4).

**Discussion**

The present study used eye-tracking technology to identify abnormal pupillary reflexes in some PD patients. These abnormal video pupillometric responses correlated with disease duration and UPDRS. These findings suggest that abnormal pupillary reflexes are a form of autonomic dysfunction seen in advanced PD.

During the study, we measured pupillary reflex responses to different stimuli, i.e., light and an isometric hand grip task. Miosis and mydriasis in response to light stimulation were significantly lower in PD patients than in control subjects (Table 2). Compared to control subjects, Miciel et al. reported a decreased amount and increased latency of miosis during the light reflex in PD patients, whereas Granholm et al. found a significantly smaller maximum rate of change in pupil diameter upon administration of 0.01% tropicamide eye drops to PD patients. These findings indicate the involvement of parasympathetic dysfunction in the poor miotic response in PD. The present study also showed a decreased miotic response (LMiR) to light stimuli in PD patients.

**Table 2.** Pupillary Responses to Each Stimulus in Patients with Parkinson’s Disease and Control Subjects.

|                     | Parkinson’s disease | Control subjects | P value |
|---------------------|---------------------|------------------|---------|
|                     | n = 45              | n = 20           |         |
| Pupil size (pixel) |                     |                  |         |
| Light stimulus      |                     |                  |         |
| Resting state       | 82.0 ± 22.1         | 80.6 ± 21.7      | 0.801   |
| Light on            | 59.1 ± 17.6         | 52.5 ± 15.2      | 0.442   |
| Light off           | 81.8 ± 21.7         | 80.6 ± 23.6      | 0.883   |
| LMiR**              | -3.29 ± 0.27        | -3.55 ± 0.13     | 0.002   |
| LMyR**              | 3.61 ± 0.42         | 3.98 ± 0.21      | 0.006   |
| Hand grip           |                     |                  |         |
| Resting state       | 78.3 ± 21.0         | 83.1 ± 22.5      | 0.415   |
| Hand grip           | 94.8 ± 23.3         | 94.8 ± 23.3      | 0.707   |
| HMyR                | 2.81 ± 0.93         | 2.52 ± 0.66      | 0.328   |

LMiR: light miosis response, LMyR: light mydriasis response, HMyR: handgrip mydriasis response.
Values are means ± standard deviation.
For LMiR, LMyR and HMyR, values are shown as log-transformed values.

**P<0.01**
Representative case of patient with Parkinson’s disease.

MIBG myocardial scintigraphy and pupillary responses in a 72 years old patient with Parkinson’s disease were shown. H/M ratios in the early and late images were severely decreased, implying the abnormality of postganglionic presynaptic cardiac sympathetic innervation. Pupillary responses in this patient also exhibited abnormal.

LMiR: light miosis response, LMyR: light mydriasis response, HMyR: handgrip mydriasis response, LEDD: levodopa equivalent daily dose.

Furthermore, poor responses in terms of LMyR were also observed in PD patients (Table 2, Figure 3). This finding differed from those of Hori et al.19 who demonstrated an excessive mydriatic response in 48% of PD patients compared to control subjects upon administration of 0.02% dipivefrin hydrochloride eye drops and Yamashita et al.20 who reported excessive mydriasis due to sympathetic denervation supersensitivity. However, the findings of a decreased mydriatic response to light in advanced PD in the present study are consistent with those of Bartošová et al.21 who showed a ceiling effect on pupil diameter at maximum mydriasis during light stimulation at a total daily dose of L-dopa of 400 mg or higher. In contrast to the reaction to light stimulation, the abnormal pupillary reflex observed in response to the isometric hand grip task was an excessive response (Table 2). Unlike the pupillary light reflex, which primarily reflects the functioning of the light reflex pathway in the midbrain12, the mydriatic response to the isometric hand grip task is evoked by activation of the sympathetic nervous system across a wide anatomical area in association with psychological stress13. Therefore, in advanced PD patients, the mydriatic response to an isometric hand grip task may reflect excessive activation of the sympathetic nerves mediated by the same pathways. This will require further research.

There are several limitations in the interpretation of the present findings. First, we did not evaluate retinal function, which serves as the input pathway for the pupillary reflex. Recent studies have identified retinal thinning at an early stage in early PD22 and reported a negative correlation between retinal thinning and disease severity22. Thus, the strength of the input signal from light stimulation may have varied among the present patients. Second, we did not compare other diseases presenting with the parkinsonian syndrome. Yamashita et al.20 investigated pupillary autonomic dysfunction in PD and multiple system atrophy (MSA) using 0.05% pilocarpine hydrochloride and 0.002% dipivefrin hydrochloride eye drops and found a significantly greater miotic response to pilocarpine eye drops in PD patients than in MSA patients and control subjects. Further investigation is required to determine the utility of eye-tracking pupillary reflexes when differentiating between PD and other diseases such as MSA-parkinsonism and progressive supranuclear palsy. Further studies are warranted to elucidate the relationship between pupillary autonomic dysfunction evaluated by means of eye drop test and our eye-tracking pupillary reflex tests.

In conclusion, abnormal eye-tracking pupillary reflex responses, such as a low response to light stimulation and a high response to the hand grip task, may be observed in PD patients. Eye-tracking video pupillometry can be used to evaluate autonomic dysfunction in PD patients. Eye-tracking video pupillometry could be used to detect preclinical or prodromal stage of Parkinson disease, which would make it a sensitive marker for establishing a preemptive therapy.

Conflicts of Interest

The authors have nothing to disclose.
Table 3. Correlation Coefficients between Pupillary Response and Clinical Variables in Parkinson’s Disease.

| Disease Duration | MDS UPDRS Part1 | Part2 | Part3 | Part4 |
|------------------|----------------|-------|-------|-------|
| LEDD             | 0.260          | 0.189 | 0.227 | 0.245 |
|                  | 0.389*         | 0.454**|
| LMIR             | -0.174         | -0.097| -0.159| -0.103|
|                  | -0.213         | -0.371*|
| LMyR             | 0.394**        | 0.333*|
|                  | -0.052         | 0.001 |
| HMyR             | 0.001          | 0.001 |

LMIR: light miosis response, LMyR: light mydriasis response, HMyR: hand grip mydriasis response, LEDD: levodopa equivalent daily dose. Log transformation was applied to transform the distribution of LMIR, LMyR, and HMyR from non-normal to normal. Values are Spearman’s correlation coefficients.

*P<0.05, **P<0.01

Table 4. Multiple Linear Regression Analyses in Parkinson’s Disease.

|                   | b    | SEb  | beta | p value |
|-------------------|------|------|------|---------|
| LMIR              |      |      |      |         |
| Age               | -0.001| 0.004| -0.020| 0.892   |
| Disease duration  | 0.018| 0.010| 0.341 | 0.083   |
| MDS-UPDRS part3   | 0.008| 0.003| 0.438 | 0.005   |
| LEDD              | 4.730×10^{-5} | 0.001| 0.064 | 0.746   |
| LMyR              |      |      |      |         |
| Age               | 0.001| 0.007| -0.003| 0.983   |
| Disease duration  | -0.037| 0.017| -0.444| 0.037   |
| MDS-UPDRS part3   | -0.006| 0.004| -0.222| 0.167   |
| LEDD              | 0.001| 0.001| 0.110 | 0.606   |
| HMyR              |      |      |      |         |
| Age               | 0.030| 0.015| 0.309 | 0.055   |
| Disease duration  | 0.029| 0.037| 0.159 | 0.436   |
| MDS-UPDRS part3   | -0.005| 0.010| -0.084| 0.592   |
| LEDD              | 0.001| 0.001| 0.211 | 0.310   |

LMIR: light miosis response, LMyR: light mydriasis response, HMyR: hand grip mydriasis response, LEDD: levodopa equivalent daily dose.

*P<0.05

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