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Pupillography refines the diagnosis of diabetic autonomic neuropathy

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Abstract

Although diabetic autonomic neuropathy involves most organs, diagnosis is largely based on cardiovascular tests. Light reflex pupillography (LRP) non-invasively evaluates pupillary autonomic function.

We tested whether LRP demonstrates autonomic pupillary dysfunction in diabetics independently from cardiac autonomic neuropathy (CAN) or peripheral neuropathy (PN).

In 36 type-II diabetics (39–84 years) and 36 controls (35–78 years), we performed LRP. We determined diameter (PD), early and late re-dilation velocities (DV) as sympathetic parameters and reflex amplitude (RA) and constriction velocity (CV) as parasympathetic pupillary indices. We assessed the frequency of CAN using heart rate variability tests and evaluated the frequency of PN using neurological examination, nerve conduction studies, thermal and vibratory threshold determination.

Twenty-eight (77.8%) patients had abnormal pupillography results, but only 20 patients (56%) had signs of PN or CAN. In nine patients with PN, only pupillography identified autonomic neuropathy. Four patients had pupillary dysfunction but no CAN or PN.

In comparison to controls, patients had reduced PD, late DV, RA and CV indicating sympathetic and parasympathetic dysfunction. The incidence and severity of pupillary abnormalities did not differ between patients with and without CAN or PN.

LRP demonstrates sympathetic and parasympathetic pupillary dysfunction independently from PN or CAN and thus refines the diagnosis of autonomic neuropathy in type-II diabetics.

Keywords: Pupillary light reflex; Autonomic nervous system; Autonomic neuropathy; Infrared pupillometry; Type-II diabetes mellitus

1. Introduction

Autonomic neuropathy is one of the most disabling complications of diabetes mellitus with symptoms such as diarrhea or constipation, hypohidrosis or excessive gustatory sweating, erectile dysfunction or orthostatic hypotension [1,2]. Several epidemologic studies demonstrate increased mortality rates in diabetic patients with autonomic neuropathy even if patients are still clinically asymptomatic [3–6]. Early diagnosis of autonomic dysfunction in still asymptomatic patients and initiation of a more intense treatment might thus contribute to preventing a progression towards overt autonomic failure [1,2,7,8]. Clinical diagnosis of autonomic dysfunction is largely based on the assessment of cardiac autonomic neuropathy by means of evaluation of heart rate variability (HRV) in response to challenge maneuvers such as metronomic breathing, Valsalva maneuver or active standing up [1,2,7,8]. These tests primarily evaluate parasympathetic autonomic function [1,9–13]. The additional evaluation of autonomic function of other organs might be relevant to identify autonomic dysfunction already in early stages of the disease.

The modulation of the pupillary diameter is known to be reduced in advanced stages of diabetes mellitus [14,15]. Changes of the pupillary diameter, e.g. in response to light stimulation, are controlled by the sympathetic as well as the parasympathetic nervous system [14,16–23]. Several studies showed associations between abnormal autonomic control of the pupils and the prevalence of peripheral [14,24] or cardiac autonomic neuropathy [15] in advanced stages of diabetes mellitus. However, the prevalence and severity of sympathetic versus parasympathetic dysfunction might be different at the eye and the heart. Diabetic neuropathy is a length-dependent dying-back neuropathy. The longer parasympathetic fibers innervating the heart are affected at

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earlier stages than the cardiac sympathetic fibers [1,9–13] while sympathetic pupillary dysfunction might be more pronounced than parasympathetic pupillary dysfunction due to greater length of sympathetic than parasympathetic fibers innervating the eye [25]. Moreover, the vulnerability of the cardiac and pupillary autonomic nerve fibers might be different in diabetes mellitus.

So far, it has not been studied whether the prevalence of pupillary autonomic dysfunction differs from that of cardiac autonomic neuropathy or peripheral somatic neuropathy.

To evaluate whether the assessment of pupillary function refines the early diagnosis of diabetic autonomic neuropathy, we studied pupillary light reflex responses in diabetic patients and compared results to the evaluation of cardiac autonomic and peripheral somatic neuropathy.

2. Materials and methods

2.1. Patients

Thirty-six type-II diabetic patients (16 female, 20 male, 39–84 years old [mean age: 57.3 ± 23.5] with mean HbA1c values of 7.45 ± 2.01 mg% and a disease duration ranging between 0.5 and 30 years [mean duration: 18.5 ± 15.8]) and 36 healthy controls (16 female, 20 male, 34–78 years old [mean age: 55.1 ± 19.7]) participated in the study. Informed consent was obtained according to the declaration of Helsinki. Patients with evidence of renal insufficiency, as defined by a serum creatinine of more than 2.2 mg/dl (194.7 mmol/l), or other organic diseases, especially cardiovascular, hepatic, pulmonary or neurological diseases, were excluded from the study. All examinations were performed between 9 AM and 2 PM. Patients were tested in a relaxed and comfortable position in a quiet room with an ambient temperature of 24 °C and stable humidity. All patients underwent a complete neurological examination and all study participants had an ophthalmologic examination. If there was pathology of the refractory media of an eye, such as corneal scarring in the main ocular axis or cataracts, or retinal as well as optic nerve damage, this eye was not included in the analysis. Moreover, no study participant has had previous cataract surgery. In 2 of the 36 patients, these exclusion criteria resulted in the analysis of pupillary function in one eye only. In all other patients, we analyzed pupillary responses of both eyes. Patients and controls did not receive any medication affecting sympathetic or parasympathetic pupillary function.

2.2. Light reflex pupillography

Pupillary function was tested after 45 min of dark-adaptation in a dim room with a background illumination of 1.25-ft candles (13.46 lx). Subjects were instructed to fixate a target point mounted on the wall of the examination room in a distance of 5 m to prevent the pupillary near response or accommodation adjustments. The pupillary diameter and light reflex responses were determined by means of a CIP 9.08™ pupillometer (AMTech, Weinheim, Germany) as previously described [26,27]. We evaluated the following static and dynamic parameters: pupil size, light reflex amplitude as well as constriction and re-dilation velocities (Fig. 1a and b). Pupil size and re-dilation velocities are parameters reflecting sympathetic pupillary modulation [17,28,29], while light reflex amplitude and constriction velocity are parameters depending on parasympathetic pupillary modulation [17,20,29]. Calculations of the pupillary parameters have been performed as described in previous studies using the same methodology and software (CIPTERM™, AMTech) [30,31]. To assure stable mean values of pupillary parameters for each participant, pupillographic measurements of each eye were taken until we obtained four artifact-free results. Parameters were calculated as the average values of these four recordings. Parameters exceeding the mean values of the control group by more than two standard deviations were considered abnormal. Sympathetic pupillary dysfunction was diagnosed when two of the three sympathetic pupillary parameters were above normal limits. Parasympathetic pupillary dysfunction was diagnosed when both, constriction amplitude and constriction velocity were abnormal.

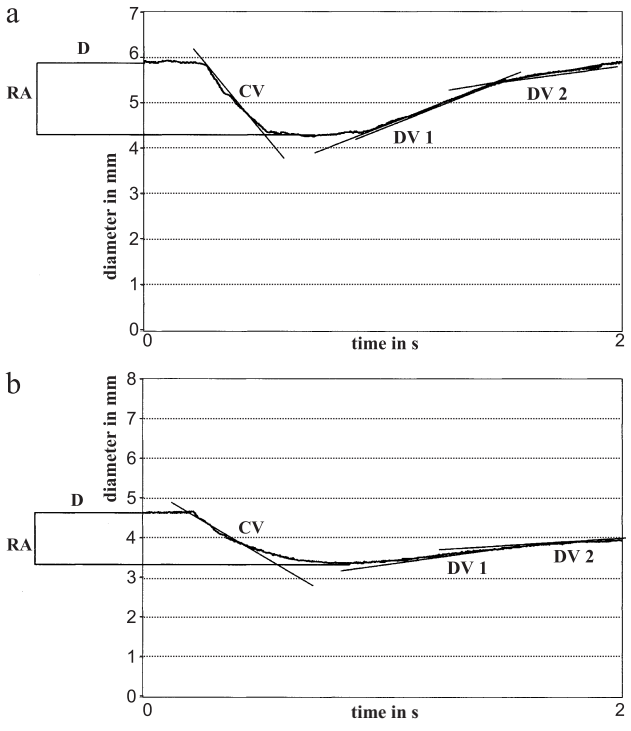

Fig. 1. The pupillary diameter changes in response to 10^4 cd light stimulation for 0.2 s and after dark-adaptation to 13.43-lx background illumination show a brisk and well-pronounced light reflex curve in a 41-year-old control person (a), but an attenuated and flattened pupillary light reflex curve in a 43-year-old diabetic patient (b) (RA = reflex amplitude; D = diameter; CV = constriction velocity; DV 1 = early re-dilation velocity; DV 2 = late re-dilation velocity).

2.3. Cardiovascular autonomic function tests

To assess the prevalence of cardiac autonomic neuropathy, we used a standardized battery of cardiovascular tests. The various tests were performed after an adequate resting phase to assure that heart rate (HR) had returned to baseline values before we started a new challenge maneuver. The electrocardiogram (ECG) signals were transferred to a personal computer via an analog–digital converter and were analyzed offline using an automatic program package (ProSciCard, MediSyst, Linden, Germany). Four tests of heart rate variability (HRV) and one test of blood pressure (BP) control were performed and evaluated according to standard criteria: recording at rest (HRV), metronomic breathing (HRV), Valsalva maneuver (HRV) and active standing up (HRV and BP) [8,12,13,32–36]. According to the criteria of Ewing [9,10], we diagnosed cardiac autonomic neuropathy when the results of three out of these five autonomic function tests exceeded age-related normal control values. According to these criteria, we classified the patients in two subgroups, patients with or patients without cardiovascular autonomic neuropathy.

2.4. Evaluation of peripheral neuropathy

2.4.1. Nerve conduction studies

In addition to the full neurological examination, all patients underwent studies of median and peroneal motor nerve conduction velocities (NCVs) and ulnar and sural sensory NCVs according to standard techniques [37] using a Viking IV™ electrophysiology system (Nicolet, Madison, WI). Age-related normal values of NCVs were taken from Ludin [38]. Amplitudes were defined as pathologic when they were below the age-dependent normal values reported by Kimura [37]. For NCV studies, the temperature of the tested skin areas was raised to 35 °C by an infrared lamp prior to the examination and was kept at this level during the tests [38].

2.4.2. Vibratory thresholds

Vibration sensation at the first metatarsal bone was tested with a hand-held electromagnetic 100 Hz Vibrameter (Somedic, Stockholm, Sweden) as previously described [39–41]. Vibratory thresholds were determined using the "method of limits" as described by Goldberg and Lindblom [39] and Hilz et al. [40,41]. In a first step, the vibration amplitude of the 13-mm diameter probe was steadily increased until the patient first perceived vibratory sensation (vibration perception threshold, VPT). In a second step, a supraliminal stimulus was reduced until the patient reported that the sensation had disappeared (vibration disappearance threshold, VDT). VPT and VDT were each determined three times. Finally, the vibration threshold, VT, was calculated as the average of mean VPT and mean VDT. Vibrameter thresholds were compared to age related normal values published by Hilz et al. [41].

2.4.3. Thermal thresholds

Warm and cold perception thresholds were also determined by psychophysical measurements using a Somedic Thermotest™ (Somedic), a modification of the "Marstock" device [42–45]. Warm and cold perception thresholds were also determined by the "method of limits" as previously described [42–45]. Thresholds were determined at four body sites: at the distal volar forearm 3 cm proximal to the wrist, at the thenar, at the distal medial calf 4–5 cm above the medial malleolus in the L4 dermatome, and at the lateral dorsum of the foot in the area innervated by the sural nerve. A 2.5×5.0-cm thermode was used for testing at the forearm, calf, and foot. A 1.5×2.5-cm thermode was used at the thenar. Temperature perception thresholds were compared to our previously published age-related normal values [44,45].

Criteria required for the diagnosis of peripheral neuropathy were adapted from Dyck [46]. They included the findings of: (1) at least two objective clinical signs (e.g. muscle weakness or decreased tendon reflexes); or (2) one clinical sign together with two or more abnormal results of nerve conduction studies; or (3) one clinical sign together with one or more abnormal nerve conduction findings, and one or more impaired vibratory or thermal thresholds; or (4) one clinical sign together with two impaired vibratory or thermal thresholds; or (5) one objective clinical or neurophysiologic sign and two subjective symptoms (e.g. paresthesias or burning feet). According to these criteria, we classified the patients in two subgroups, patients with and patients without peripheral neuropathy.

2.5. Statistics

For statistical analysis, we used a commercially available program (SYSTAT, Evanston, IL). The two-sided Mann–Whitney *U*-test was used to compare the pupillography results of patients and controls. The level of significance was set at $p \leq 0.05$. Pupillography results were correlated with the age of the patients, the duration of diabetes, the HbA1c levels, the nerve conduction studies, the vibratory, thermal and cardiovascular test results using the Spearman rank correlation test.

3. Results

Fig. 2 demonstrates the number of patients with abnormal results of pupillary light reflexes alone and in combination with test results of cardiovascular autonomic function and/or peripheral nerve function. Overall, light reflex pupillography showed abnormal results in 28 of our 36 patients (77.8%). Seven of the 36 patients (19.4%) showed abnormal results with the tests of cardiac autonomic and/or peripheral neuropathy. Only 1 of the 36 patients (2.8%) had normal results with all of the three procedures testing for pupillary dysfunction, cardiac autonomic neuropathy and peripheral somatic neuropathy.

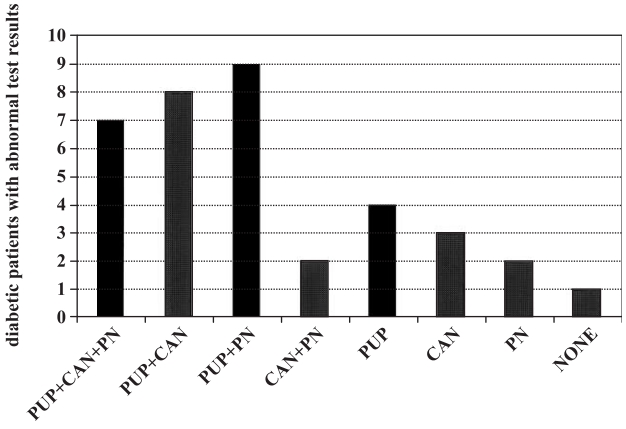

Fig. 2. Prevalence of abnormal results with procedures testing for pupillary light reflex abnormalities (PUP), cardiovascular autonomic neuropathy (CAN) and peripheral neuropathy (PN). In 9 of the 36 patients (25%) with signs of peripheral neuropathy, only pupillography revealed additional autonomic neuropathy. In 4 of the 36 patients (11.1%), only pupillography detected abnormal nerve function while testing for cardiovascular and peripheral neuropathy showed normal results.

3.1. Light reflex pupillography

Pupillary parameters were significantly smaller in patients than controls ($p < 0.05$, Table 1). In the controls, light reflex responses were brisk and well pronounced (Fig. 1a). In the patients, response curves were attenuated and flattened (Fig. 1b).

The baseline pupillary diameter was significantly smaller in the patients (4.84 ± 0.98 mm) than in the controls (5.91 ± 1.08 mm; $p < 0.05$; Table 1). Moreover, both re-dilation velocities were reduced in the patients (Table 1). The early re-dilation velocity was slower in the diabetics (1.19 ± 0.54 mm/s) than in the controls (1.51 ± 0.53 mm/s; $p < 0.05$; Table 1). Similarly, the late re-dilation velocity was smaller in the diabetics (0.52 ± 0.22 mm/s) than in the controls (0.72 ± 0.37 mm/s; $p < 0.05$; Table 1), although the difference was less pronounced. The light reflex amplitude was also significantly smaller in the patients (1.30 ± 0.36 mm) than in the controls (1.55 ± 0.31 mm; $p < 0.05$) and the maximum constriction velocity was slower in the patients (3.90 ± 1.36 mm/s) than in the controls (4.49 ± 0.96 mm/s; $p < 0.05$; Table 1).

In 21 patients, all pupillography parameters were abnormal indicating sympathetic and parasympathetic pupillary dysfunction. In seven patients, there was a reduction of the initial pupillary diameter and of early and late dilation velocities indicating sympathetic pupillary dysfunction only.

3.2. Light reflex pupillography in diabetics with peripheral neuropathy compared to diabetics without peripheral neuropathy

Twenty of the 36 patients (56%) were classified as having polyneuropathy according to the above criteria. Neither the frequency of abnormal pupillography results (Fig. 2) nor the values of the pupillary light reflex parameters (Table 1) differed between patients with and those without peripheral neuropathy ($p > 0.05$).

3.3. Light reflex pupillography in diabetics with and without CAN

Twenty patients (56%) had CAN according to the criteria suggested by Ewing et al. [9,10]. Six of the 20 patients (30%) had abnormal results with three of the five cardiovascular function tests. In 13 patients (65%), even four of the tests showed abnormal results. One patient (5%) had additional orthostatic hypotension, i.e. results of all the five cardiovascular tests were pathologic. Again, the frequency of abnormal light reflex results or pupillary light reflex values did not differ between patients with and those without cardiac autonomic neuropathy (Table 1; Fig. 2; $p > 0.05$).

The age of patients, the duration of diabetes or the glycemic control did not differ between the patients with

Table 1
Pupillary light reflex parameters in 36 type-II diabetic patients and 36 controls

	Diabetics	Controls	*U*-test	Diabetics		*U*-test	Diabetics		*U*-test
				With	Without		With	Without	
				Peripheral somatic neuropathy			Cardiac autonomic neuropathy		
n	36	36		20	16		20	16	
Sympathetically mediated pupillary light reflex parameters									
Pupillary diameter (mm)	4.84 ± 0.98	5.91 ± 1.08	$P = 0.04$	4.52 ± 0.91	5.17 ± 0.89	$P = 0.09$	4.72 ± 0.87	4.95 ± 0.96	$P = 0.07$
Early re-dilation velocity (mm/s)	1.19 ± 0.54	1.51 ± 0.53	$P = 0.01$	1.09 ± 0.47	1.34 ± 0.61	$P = 0.12$	1.08 ± 0.44	1.42 ± 0.70	$P = 0.22$
Late re-dilation velocity (mm/s)	0.52 ± 0.22	0.72 ± 0.37	$P = 0.02$	0.51 ± 0.19	0.52 ± 0.24	$P = 0.18$	0.53 ± 0.17	0.41 ± 0.17	$P = 0.22$
Parasympathetically mediated pupillary light reflex parameters									
Reflex amplitude (mm)	1.30 ± 0.36	1.55 ± 0.31	$P = 0.02$	1.25 ± 0.36	1.37 ± 0.37	$P = 0.12$	1.28 ± 0.35	1.33 ± 0.34	$P = 0.13$
Constriction velocity (mm/s)	3.90 ± 1.36	4.49 ± 0.96	$P = 0.001$	3.76 ± 0.96	3.79 ± 0.87	$P = 0.25$	3.67 ± 0.95	3.88 ± 0.79	$P = 0.09$

Significant differences between pupillary light reflex parameters in 36 type-II diabetic patients and 36 controls (*U*-test: $p < 0.05$). Pupillary parameters did not differ between patients with and without peripheral neuropathy nor between patients with and without cardiac autonomic neuropathy.

and without peripheral neuropathy nor between those with and without cardiovascular autonomic neuropathy (p-values >0.05 each). Moreover, there were no significant correlations between the results of infrared pupillography and age of the patients ($r_s = 0.34$), duration of diabetes ($r_s = 0.28$), the quality of diabetes control ($r_s = 0.40$), the nerve conduction studies, the vibratory and thermal testing or the cardiovascular test results ($r_s < 0.5$).

4. Discussion

Our results demonstrate that impairment of light reflex responses occurs independently from peripheral diabetic neuropathy or from cardiovascular autonomic neuropathy. The prevalence of pupillary autonomic dysfunction was similar in patients with and those without signs of cardiac autonomic or peripheral neuropathy. Moreover, there is no correlation between parameters of cardiovascular and pupillary testing. More than 11% of our patients (4/36) had abnormal pupillary autonomic function but no signs of cardiac autonomic or peripheral neuropathy. In an additional 25% of our patients (9/36), we would have diagnosed peripheral neuropathy but would have missed autonomic neuropathy without light reflex testing.

Obviously, pathology of pupillary nerve fibers may develop independently from the cardiac autonomic or peripheral neuropathy. This assumption is supported by the observation that cardiovascular autonomic neuropathy sometimes also develops independently from somatic neuropathy [47,48]. The dying-back pathology in diabetic neuropathy suggests that cardiac parasympathetic neuropathy should manifest prior to pupillary dysfunction since the distal part of the rather long vagal nerve should be more vulnerable than the relatively short fibers innervating the pupils [49]. Still, several of our patients presented with deficient pupillary innervation but had no signs of somatic or cardiac neuropathy. We speculate that pupillary autonomic innervation might be more vulnerable to diabetic pathology than peripheral nerve fibers. Oculomotor or trochlear neuropathy is known to occur independently from peripheral or cardiac autonomic neuropathy [49–51]. Moreover, the innervation of ciliary and iris muscles is highly selective, i.e. the number of muscle fibers innervated by a single nerve fiber is low [25]. Consequently, subtle dysfunction of pupillary nerve fibers may already result in clinically manifest pupillary dysfunction before the onset of cardiac or peripheral neuropathy.

Abnormal light reflex responses might be due to a dysfunction at any level of the afferent and efferent branches or at the centers of central processing of the light reflex arc [17,18,52]. In our patients, an afferent dysfunction seems to be a less likely cause of the abnormal results as we excluded patients with refractory pathologies such as corneal scarring and pathology of the corpus vitreum, retinopathy and optic nerve atrophy from the study. Hence, abnormalities are more likely to reflect pathology at the level of the central processing pathways or the efferent sympathetic and parasympathetic branches of the pupillary light reflex arc [17,18,52].

In 21 patients, all light reflex parameters were below normal limits showing that there was a dysfunction of sympathetic and parasympathetic pupillary innervation.

Smith and Smith [22] conclude that slow pupillary constriction velocities and decreased reflex amplitudes in diabetic patients cannot be ascribed to small pupillary diameters at rest but are due to a parasympathetic dysfunction. Hayashi and Ishikawa [53] provided evidence of parasympathetic pupillary denervation in diabetic patients by demonstrating supersensitive pupillary responses to cholinergic drugs. Reduction of the resting pupillary diameter and the re-dilation velocities, indicating impaired sympathetic pupillary modulation, was not only present in these 21 patients but also in 7 additional patients. According to Smith et al. [28,29], the resting diameter is mainly under sympathetic control and the diameter reduction is a sign of diminished sympathetic outflow to the iris muscles. Similarly, the slowing of the early and late re-dilation velocities is a parameter of deficient sympathetic pupillary modulation [28,29]. The early re-dilation velocity depends on parasympathetic withdrawal and sympathetic activation while the late re-dilation velocity is exclusively mediated by sympathetic outflow [28,29]. Smith and Smith [29] showed sympathetic pupillary denervation in patients with diabetic autonomic neuropathy by means of pharmacological testing. Moreover, the authors demonstrated a prompt and pronounced pupillary dilation with instillation of sympathomimetics and concluded that impairment of pupillary responses was not due to a direct muscular dysfunction of the iris [29]. Our finding of more frequent sympathetic (28/28) than parasympathetic pupillary dysfunction (21/28) is in accordance with previous light reflex studies by Smith et al. [14,15] and with the clinical observation of small pupils in diabetic patients [14,22–24].

The higher prevalence of sympathetic pupillary abnormalities can be attributed to the above-mentioned dying-back pathology in diabetic neuropathy [49–51]. The sympathetic pupillary fibers are longer than the parasympathetic fibers and therefore seem to be more vulnerable than the oculomotor fibers [49–51]. In irides removed during cataract surgery in diabetic patients, loss of nerve terminals occurs mostly from the sympathetically innervated dilator pupillae [54].

To summarize, light reflex pupillography reveals abnormal pupillary function even in diabetic patients without signs of peripheral or cardiac autonomic neuropathy. As pupillography might identify autonomic dysfunction earlier than standard tests of cardiac autonomic neuropathy, the technique should complement methods assessing peripheral or cardiac autonomic neuropathy. Our data show that light reflex pupillography refines the early diagnosis of autonomic dysfunction.

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