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Evaluation of pupillary response to light in patients with glaucoma: a study using computerized pupillometry

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Abstract The aim of this study was to evaluate pupillary response to light stimulation in patients with different stages of glaucoma using computerized pupillometry. We conducted a retrospective study on a group of 44 glaucoma patients who had undergone complete ophthalmological examination, visual field test (Humphrey SITA Standard 24-2) and monocular dynamic pupillometry (MonCV3 Metrovision). Eyes were classified into stages of glaucoma according to visual field damage using the Glaucoma Staging System 2. A group of 18 healthy subjects, homogeneous for age and sex with glaucoma patients, was used as a control. The following parameters were considered—latency and duration of contraction and dilatation; initial, minimum, maximum, and mean pupil diameter; amplitude of contraction; contraction and dilatation speed; and percent pupil contraction (PPC). PPC and pupil contraction speed and minimum diameter showed covariate correlation with the stages of glaucoma. The control group significantly differed from the stage 3 group in terms of PPC and from the stage 4 group in terms of minimum diameter. There were significant differences between the stage 5 group and stage 1, 2, 3 and control groups. Ordinal logistic regression showed a correlation between pupil contraction speed, minimum diameter, PPC, initial diameter and the stage of glaucoma. The study showed that glaucoma damage is associated with altered values of pupillary response to light. This event may be the consequence of the progressive loss of retinal ganglion cells and their axons induced by glaucoma.

Keywords Glaucoma · Pupillary light reflex · Pupillometry · Visual field

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Introduction

The pupil light reflex (PLR) is a four-neuron arc that controls the pupil diameter in response to the light that falls on the retina, thereby assisting in adaptation to various levels of darkness and light. This physiological reaction is routinely elicited during the ophthalmological examination as a functional marker of the retina, the optic nerve, and the brain stem [1]. PLR abnormalities usually manifest as a relative afferent pupillary defect and depend on a variety of conditions that involve the integrity of the entire visual pathway such as glaucoma [2–5].

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Table 1 Demographic characteristics of the eyes included in the study according to control and glaucoma groups

Characteristics	Control group	Glaucoma groups				
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
No. of patients	18	44				
Males/females	13/8	5/11	11/5	8/8	10/5	11/5
Age (years) (mean ± SD)	63.2 ± 10.2	67.1 ± 7.2	68.5 ± 5.8	69.1 ± 3.2	65.8 ± 4.7	70.8 ± 4.1
No. of eyes	21	16	16	16	15	16
No. of discarded eyes	15	9				

Glaucoma is a leading cause of blindness worldwide [6, 7]. It is characterized by progressive degeneration of retinal ganglion cells (RGCs) and loss of their axons which make up the retinal nerve fiber layer and then aggregate to form the optic nerve. The recent observation that rats with experimental glaucoma suffer from PLR deficit correlated with intraocular pressure (IOP) elevation suggests that monitoring PLR alterations may be a useful and objective test in the detection of retinal and optic nerve deficits in glaucoma [8]. Several studies evaluated the importance of PLR assessment in patients with this disease. Measurement of pupil response using infrared pupillography was introduced by Lowenstein and Loewenfeld in 1958 and efforts were later made to develop automated pupil perimetry, an approach used to quantify monocular pupillary responses [9]. Studies in patients with asymmetric glaucoma damage between the upper and lower retina demonstrated that light stimulations projected separately to these areas produced different PLR responses [10] and that altered PLR may also be elicited using a stripe pattern offset–onset stimulus [11].

Accordingly, our aim was to evaluate the changes of pupillary response to light stimulation in patients affected by different stages of glaucoma using computerized pupillometry, in order to find clinically useful parameters in glaucoma diagnosis and follow-up.

Materials and methods

We conducted a retrospective study on 62 patients (18 controls and 44 glaucoma patients) attending the Ophthalmological Department of University Hospital Tor Vergata who underwent dynamic monocular pupillometry examination. Table 1 shows demographic characteristics of the eyes included in the study according to control and glaucoma groups. Values for the pupillometric parameters of the glaucomatous and control eyes are shown in Table 2.

The medical history of each patient was reviewed to exclude those with eye or systemic diseases known to affect pupillary motility, such as secondary open-angle glaucoma, pigment dispersion syndrome [12], pseudoexfoliation syndrome, intraocular surgery or laser treatments and, for the same reason, thyroid disorders, diabetes or neurological diseases [1]. Patients receiving systemic or topical medications that could affect iris mechanics, such as brimonidine [13], pilocarpine, and narcotic-derived medications for pain control were also excluded from the study.

All patients included in the study must have undergone a comprehensive ophthalmological examination, a monocular computerized pupillometry and reliable visual field (VF) testing within 2 months prior to the study. Furthermore, each patient must have a best-corrected visual acuity of 0.0 logMar, no alterations of the anterior segment at the slit-lamp examination, and no previous cataract surgery.

The diagnosis of glaucoma was based on the Melbourne Visual Impairment Project criteria—a past history of primary open-angle glaucoma, IOP > 21 mmHg, VF defects including enlarged blind spot, cup-to-disc ratio (CDR) of ≥0.7 and/or asymmetry of vertical CDR of ≥0.3 [14].

VF tests must have been assessed using Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) and the SITA (Swedish Interactive Threshold Algorithm) standard 24–2 program. VF tests with false–negative, false–positive, and fixation losses >30 % were considered unreliable and relevant eyes were discarded (Table 1) [15].

Table 2 Pupil measurements in control and glaucoma groups

Characteristics	Control group	Glaucoma groups				
		Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Percent pupil contraction	37.95 ± 8.09	36.30 ± 5.44	31.20 ± 8.35	29.57 ± 7.31	32.02 ± 6.02	32.90 ± 5.15
Amplitude of pupil contraction (mm)	1.205 ± 0.435	1.950 ± 2.435	1.094 ± 0.391	0.975 ± 0.317	1.173 ± 0.363	1.056 ± 0.329
Latency of pupil dilatation (ms)	768.24 ± 141.66	833.44 ± 80.78	774.88 ± 113.96	762.63 ± 98.09	784.53 ± 139.61	822.94 ± 120.38
Latency of pupil contraction (ms)	309.57 ± 58.836	339.56 ± 30.481	302.06 ± 62.669	310.50 ± 48.284	315.60 ± 64.053	331.31 ± 71.597
Duration of pupil contraction (ms)	458.71 ± 98.884	506.25 ± 87.804	472.88 ± 112.285	556.19 ± 198.811	495.53 ± 117.383	500.06 ± 133.960
Pupil contraction speed (mm/s)	4.228 ± 1.554	4.394 ± 1.050	3.963 ± 1.190	3.910 ± 0.889	3.935 ± 1.189	2.570 ± 0.826
Pupil dilatation speed (mm/s)	1.849 ± 0.801	1.724 ± 0.391	1.671 ± 0.573	1.644 ± 0.392	1.527 ± 0.312	1.491 ± 0.275
Duration of pupil dilatation (ms)	1,603.19 ± 345.44	1,647.94 ± 82.47	1,654.13 ± 109.54	1,591.56 ± 215.50	1,671.13 ± 119.49	1,618.75 ± 159.51
Initial pupil diameter (mm)	3.962 ± 0.505	4.225 ± 0.493	4.119 ± 0.742	4.081 ± 0.597	4.380 ± 0.672	4.125 ± 0.565
Maximum pupil diameter (mm)	4.748 ± 0.685	4.944 ± 0.694	4.900 ± 0.821	4.963 ± 0.623	4.920 ± 0.787	4.694 ± 0.689
Minimum pupil diameter (mm)	2.448 ± 0.374	2.681 ± 0.308	2.825 ± 0.591	2.863 ± 0.413	2.973 ± 0.524	2.756 ± 0.348
Mean pupil diameter (mm)	3.51 ± 0.44	3.79 ± 0.42	3.76 ± 0.65	3.83 ± 0.51	3.97 ± 0.60	3.76 ± 0.47

Patients were divided into five groups according to the severity of the VF alterations using Glaucoma Staging System 2 [16]. Patients at the borderline stage were not included in the study to overcome possible overlapping between glaucoma and control groups.

Monocular dynamic pupillometry (MonCV3 Metrovision) must have been performed in both eyes (one eye at a time by occluding one eye) in darkness after 5 min of darkness adaptation, for a duration of 90 s. Patients must have been examined using white light flashes (stimulation ON time 200 ms, stimulation OFF time 3,300 ms, total luminance 100 cd/m^2, total intensity 20 cd.s/m^2).

The stimulator is equipped with near-infrared illumination (880 nm) and a high-resolution near-infrared image sensor which allows measurement of pupil diameter even in complete darkness. The images of the eyes are acquired and processed in real time (30 images per second). The proprietary analysis software provided in the pupillometer automatically outlines pupillary contour on the images, ensuring the accuracy of the measurements (accuracy = 0.1 mm) under controlled illumination conditions. It then performs an analysis of the temporal and average response to successive visual stimuli with automated quantification of the following parameters—latency and duration of contraction and dilatation expressed in seconds; initial, minimum, maximum, and mean pupil diameter expressed in millimetres; the amplitude of contraction expressed in millimetres; and contraction and dilatation speed of the pupil expressed in millimetres per second.

A recent study suggested that iris mechanics limit the amount of pupil contraction and suggested that percent pupil contraction (PPC) was a parameter least influenced by this factor [13]; therefore, PPC was also evaluated.

A group of 18 healthy subjects, homogeneous for age and sex with the glaucoma group, served as a control (stage 0). Subjects were consecutively extrapolated from routine ophthalmological clinical records and must have normal IOP (<21 mmHg), normal optic disc, no findings of any ocular disease and no first-degree relatives with a history of glaucoma. Only eyes with a normal VF documented by reliable examinations were included in the study (Table 1).

To overcome the influence of daytime variations on the results, all eyes included in the study should have undergone pupillometry between 9.00 am and 1.00 pm.

This study adhered to the Declaration of Helsinki and the protocol was approved by the Institutional Review Boards and Ethics Committees of the University Hospital Tor Vergata.

Statistics and mathematical analyses

All data were initially entered into an EXCEL database (Microsoft, Redmond, WA, USA) and the analysis was performed using the Statistical Package for the Social Sciences Windows, version 15.0 (SPSS, Chicago, IL, USA).

Descriptive statistics consisted of the mean ± SD for parameter with Gaussian distributions (after confirmation with histograms and the Kolgomorov–Smirnov test), median and range (minimum/maximum) for frequencies and variables categorical with non-Gaussian distributions.

Comparison of stage among groups was performed with ANOVA/ANCOVA (and multiple comparison by Bonferroni test) for continuous parametric variables, Kruskal–Wallis (groups > 2) or Mann–Whitney (groups $= 2$) for non-parametric variables and the Chi squared test or Fisher's exact test (if <5 cells) for categorical variables.

Logistic ordinal regression was used to assess the association between pupillometric parameters and stage.

A p value of <0.05 was considered statistically significant.

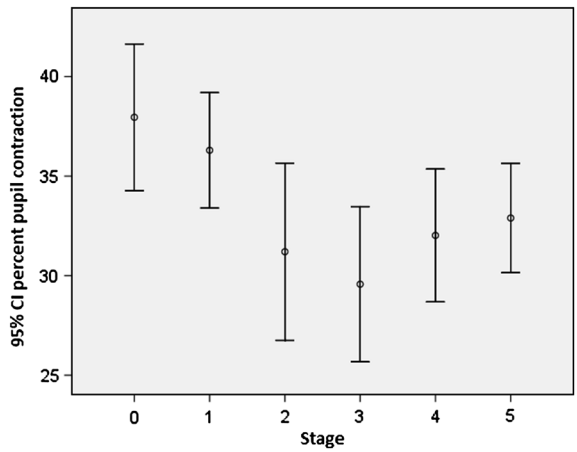

Fig. 1 Percent pupil contraction values trend. *CI* confidence interval

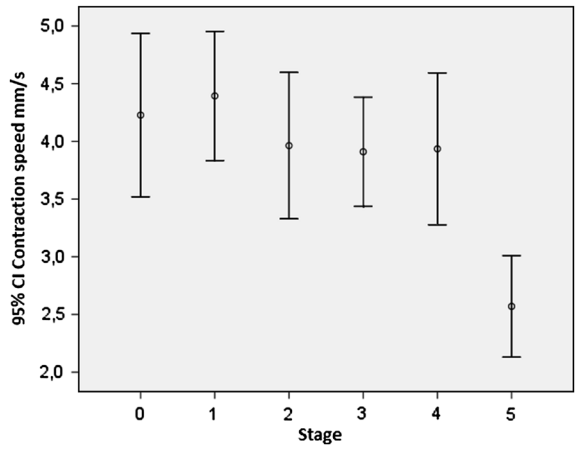

Fig. 2 Contraction speed values trend. *CI* confidence interval

Results

Statistical analysis showed a significant association between PPC and the stage of the disease ($p = 0.011$). No significant correlation could be found between PPC and sex ($p = 0.462$) and age ($p = 0.205$). The post hoc comparisons revealed a significant difference between stage 0 and 3 ($p = 0.032$). In particular, it was also observed that PPC had a tendency to decrease between stages 0 and 3 but showed an increasing trend between stages 3 and 5 (Fig. 1).

A significant correlation between pupil contraction speed and the stage of the disease ($p = 0.001$) was noticed and no associations with sex ($p = 0.697$) and age ($p = 0.313$) could be found. The post hoc comparisons revealed a significant difference between pupil contraction speed at stage 5 versus stages 0 ($p = 0.003$), 1 ($p < 0.0001$), 2 ($p = 0.020$) and 3 ($p = 0.021$). A nearly significant difference was also found between stage 5 and stage 4 ($p = 0.051$). In particular, pupil contraction speed seemed to have a tendency to become smaller with progression of the disease (Fig. 2).

A statistically significant association with stage was also found when minimum diameter was considered ($p = 0.019$) and the latter was not influenced by age ($p = 0.963$) and sex ($p = 0.154$). The post hoc comparisons showed a significant difference between stage 0 and stage 4 ($p = 0.016$); except for stage 5, this parameter seemed to increase with the progression of glaucoma (Fig. 3).

No other pupillometric parameter was found to be significantly associated with the stage of the disease;

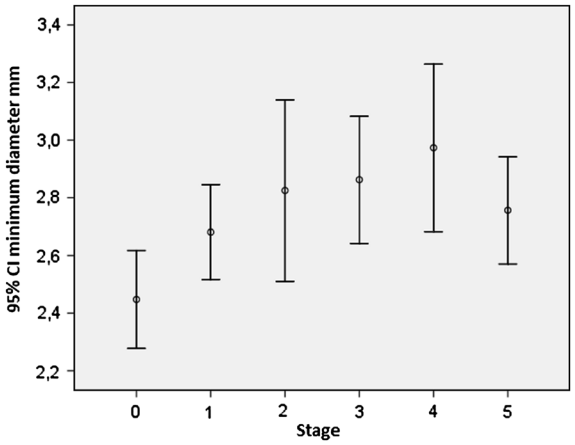

Fig. 3 Minimum diameter values trend. *CI* confidence interval

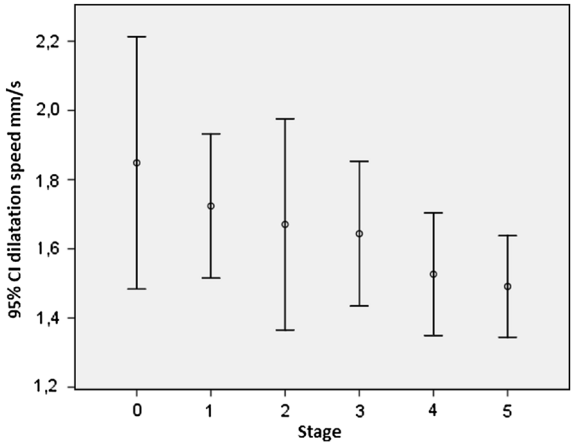

Fig. 4 Dilatation speed values trend. *CI* confidence interval

however, it was observed that dilatation speed values had a tendency to decrease with progression of glaucoma (Fig. 4).

Given the results of the statistical analysis, PLUM (ordinal regression) was used to investigate which parameter correlated best with the stage of the disease (Table 3). Pupil contraction speed showed the best correlation ($p = 0.000$) followed by minimum pupil diameter ($p = 0.002$), PPC ($p = 0.003$) and initial pupil diameter ($p = 0.004$).

Discussion

The analysis of pupillary reaction to light exposure was considered an objective evaluation of the integrity of the afferent visual pathways and of the sympathetic and parasympathetic systems. However, in accordance with a large population study by Hennessy et al. [17], test duration, complexity, operator dependency and fluctuations of the pupillary responses to changes in external ambient lightening have limited the clinical application of PLR in glaucoma. Kalaboukhova et al. [18] previously conducted experiments on the use of the mean values of pupillary area ratio (PAR), pupillary contraction velocity ratio and pupillary dilation velocity ratio (PDVR) using a non-commercial, custom-built pupillometer and reported a significant difference in PAR and PDVR values between glaucoma and control groups.

The aim of the present study was to overcome the main issues in the clinical use of PLR and to evaluate for the first time stage by stage, the alterations of the pupillary response to light induced by glaucoma using a commercial computerized pupillometer.

A pupillometer automatically provides multiple numerical indexes of pupillary response to light under controlled ambient lightening conditions, improving the repeatability of the examinations, solving the operator dependency issue and reducing the false–negative responses.

Our results suggest that among all the considered parameters, PPC, pupil contraction speed and minimum diameter seem to have a covariate correlation with the stages of the disease. In particular, stage 0 significantly differs from stage 3 in terms of PPC and from stage 4 in terms of minimum diameter. Pupil contraction speed, however, showed a significant difference between stage 5 and stages 0, 1, 2 and 3 as well as an almost significant difference between stage 5 and stage 4. Moreover, these correlations have been confirmed by ordinal logistic regression that also revealed a correlation between initial diameter and stage of the disease. This analysis has also shown that pupil contraction speed was the parameter that best fitted the stage of the disease.

The mechanisms responsible for the observed changes are still not completely known. Glaucoma is a progressive disease characterized by the apoptotic death of RGCs triggered by different molecular pathways and is also involved in neuronal damage at the level of the afferent pathway and the central visual areas [19–21]. Recent studies in rodents and primates described a new subgroup of intrinsically photosensitive retinal ganglion cells (ipRGCs), whose

Table 3 PLUM (ordinal regression)

Stage function	Estimate	SD	Wald	df	Sig.
Pupil contraction speed (mm/s)	−1.585	0.313	25.644	1	< 0.0001
Minimum pupil diameter (mm)	−19.984	6.491	9.477	1	0.002
Percent pupil contraction	−0.797	0.266	8.995	1	0.003
Initial pupil diameter (mm)	13.137	4.518	8.454	1	0.004
Latency of pupil dilatation (ms)	0.008	0.004	3.081	1	0.079
Duration of pupil contraction (ms)	−0.006	0.003	2.747	1	0.097
Mean pupil diameter (mm)	2.139	2.459	0.756	1	0.385
Maximum pupil diameter (mm)	0.686	0.858	0.64	1	0.424
Latency of pupil contraction (ms)	−0.004	0.006	0.344	1	0.558
Amplitude of pupil contraction (mm)	−0.119	0.261	0.207	1	0.649
Duration of pupil dilatation (ms)	−0.001	0.003	0.168	1	0.682
Pupil dilatation speed (mm/s)	0.09	0.711	0.016	1	0.899

contribution to the pupil light reflex has been characterized [22]. This subset of RGCs expresses melanopsin, an opsin-like protein. It projects to brain nuclei involved in non-image-forming visual functions such as PLR and circadian photoentrainment in a substantially different way from rods and cones complementing their function in mammalian vision [23, 24]. Different experimental models confirmed that ipRGCs are damaged in glaucoma [25–28], suggesting that the modification of the PLR reported in our study may be consequent to the loss of RGCs, specifically of the photosensitive subtype.

It is important to notice that pupil responses via ipRGCs may be elicited preferably using short wavelength blue light (480 nm) at high stimulus intensity and they appear to signal in brighter light and over a longer duration than the rod and cone systems [24, 29]. Therefore, through the use of white light, due to its wide range of wavelength extending from 400 nm (violet) to 700 nm (red), they are also likely to be activated in the present study.

Taken together, these findings document a pupillary ability to react to light stimulation significantly impaired by glaucoma at some stages of the disease. However, it is not possible to understand whether the ipRGCs are preferentially damaged or if there is a generalized RGC loss and how this reflects to the PLR.

If the data from our study is confirmed and a significant difference between all the stages of the disease is demonstrated by other prospective studies on larger samples of patients, then pupillometry could represent a rapid, useful and additional objective tool to detect and monitor glaucoma especially in patients without compliance with clinical tests such as VF.

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