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Pupillometry: An objective test to assess endocular hereditary transthyretin amyloidosis

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Abstract

Objective: To automatically study the pupillary light reflex in patients with hereditary transthyretin-associated amyloidosis (hATTR).

Methods: Prospective cross-sectional observational study in patients with hATTR with unilateral scalloped iris. Pupillary light reflex of scalloped iris eyes (21 eyes) were compared with non-scalloped iris eyes (21 eyes, paired eyes of the same patients) and also with a control group of 20 healthy eyes, using static and dynamic pupillometry with the Metrovision® MonPack One.

Results: No patient presented evident neurological involvment of the cranial nerves. No significant differences were found in the pupillary diameters under standardized lighting conditions (static pupillometry) among groups. In dynamic pupillometry, the amplitude of contraction, the velocity of contraction and the velocity of dilation were statistically significantly lower in eyes with scalloped iris, comparing both with the contralateral non-scalloped iris eyes ($p < 0.001$ for all) and with eyes from healthy subjects ($p < 0.05$ for all).

Conclusion: A scalloped iris reflects a more advanced endocular hATTR and it is associated with an altered pupillary light reflex. Pupillometry may be a quick, simple, and portable test to objectively evaluate ocular amyloid deposition in hATTR eyes. Pupillary light reflex may not be reliable to evaluate neurological dysfunction in these patients.

Keywords

hATTR, scalloped iris eyes, iris stiffness, pupillometry, oculopathy

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Introduction

Amyloidoses are a group of diseases which result from the accumulation of a non-soluble protein in the extracellular space of several tissues. Hereditary forms of amyloidosis result from inherited protein mutations. Hereditary transthyretin amyloidosis (hATTR),[1] an autosomal dominant disease, is caused by a mutant transthyretin (TTR). So far, more than 100 amyloidogenic TTR mutations have been documented[2,3] and several different phenotypes of TTR amyloidosis have been described, however the most frequent and widely disseminated is the substitution of valine for methionine at position 30, and Portugal remains one of the most prevalent geographic places with this variant.[4] Hereditary transthyretin amyloidosis manifests as a progressive neurodegenerative disease, characterized by the accumulation of TTR in the peripheral nerves and other organs,[5,6] including the eye.[7,8] The disease shows high phenotypic heterogeneity and variable age of onset.[9–11]

Liver transplantation has revolutionized the prognosis of the disease. This treatment replaces circulating mutant TTR with structurally normal TTR, thus increasing the

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quality and average life expectancy of these patients.[12,13] Tafamidis, a highly specific TTR stabilizer, remains the only medicine approved for the treatment of hATTR. One study showed that in a small cohort of patients taking tafamidis orally, this molecule was detected in low concentrations in the vitreous body, revealing that it could pass the eye-blood barrier and act locally.[14]

However, the intraocular production of TTR by retinal and ciliary pigment epithelial cells remains unchanged after both liver transplantation and tafamidis, making oculopathy an increasingly cumulative feature in the course of the disease.[15–17] Common ocular manifestations include abnormal conjunctival vessels, keratoconjunctivitis sicca, neurotrophic corneal ulcers, glaucoma due to progressive resistance of aqueous humor outflow in trabecular meshwork, vitreous opacities and retinal angiopathy.[18–22]

Additionally, one of the most frequent ocular features of the disease is the deposition of amyloid on the anterior surface of the lens, on the iris and on the pupillary border, leading to irregular pupils with particular indentations, named scalloped pupils.[20,21]

Ocular involvement of hATTR patients has a typical and progressive evolution (amyloid accumulates in the pupillary border, then in the anterior capsule of the lens, after that scalloped pupils become evident and finally glaucoma develops).[19,21] Due to its asymmetric progression, one patient may present with one eye with scalloped iris and the contralateral one without. This asymmetrical deposition of amyloid has prognostic implications: several studies have associated the presence of scalloped iris with the development of glaucoma.[21–23] Others revealed that the deposition of amyloid in the anterior lens capsule and pupil cause poor quality of vision by reducing contrast sensitivity and causing early presbyopia.[24,25]

Pupillary abnormalities in these patients could also be explained by the progressive autonomic neuropathy.[26,27] However, cranial nerve involvement in hATTR with V30M mutation is typically a late finding.[28]

Automatic pupillometry is a non-invasive, non-contact, safe, and fast clinical validated exam to measure the pupillary light reflex.

Therefore, the aim of this study was to analyze the pupillary light reflex, with automatic pupillometry, in patients with hATTR.

Methods

Study design and population

Observational cross-sectional study in the group of patients with hATTR due to V30M mutation (with both genetic and histopathological confirmation), followed in *Centro Hospitalar e Universitário do Porto*, the Portuguese reference center for hATTR. Even without ophthalmologic symptoms, these patients are sent to an annual eye screening.

From January 2016 to December 2018, we consecutively included 21 hATTR patients (42 eyes) that exhibited unilateral scalloped iris (one eye with scalloped iris and the contralateral eye without scalloped iris). An age-matched control group of 10 healthy subjects (20 eyes) from the routine ophthalmology clinic was also included.

Exclusion criteria comprised other abnormalities of the iris, such as areas of atrophy, dialysis, nodules, cysts, corectopia or aniridia, and patients with previous ocular surgery.

Automatic pupillometry

Statis and dynamic pupillometry were performed in all subjects with the Metrovision® MonPack One ocular electrophysiology equipment. This system is equipped with a near infra-red illumination and a high-resolution camera that allows measurements of bilateral pupils.

Static pupillometry measures pupillary diameters under standardized levels of illumination (high photopic – 100 candle/meter^2 (cd/m^2), low photopic – 10 cd/m^2, mesopic – 1 cd/m^2 and scotopic 0.1 cd/m^2). The system provides accurate measurements of diameters (accuracy of 0.1 mm) and the pupil is outlined on the image allowing validation by the investigator. A visual example is shown in Figure 1.

In dynamic pupillometry, the images of both eyes were acquired and processed in real time (30 images per second) and the average response to the successive visual stimuli (light flashes) was quantified in the following parameters: amplitude of contraction and velocity, latency and duration of both contraction and dilatation. The values considered are the mean of three measurements.

Protocol and statistical analysis

Outcomes were compared among the scalloped iris eyes (group 2), the non-scalloped iris eyes of hATTR patients (group 1) and the eyes of healthy subjects (group 0). Paired *t*-tests were used to compare groups 1 and 2 (eyes from the same hATTR patient) and independent sample *t*-tests were used to compare with group 0 (eyes from healthy subjects). A full neurological and ophthalmological examination were also performed.

A two-sided p-value of <0.05 was considered statistically significant. Cohen's d effect sizes and 95% confidence intervals were calculated to assess the magnitude of those differences. Differences were regarded as meaningful but small at Cohen's d of 0.20, medium at 0.50, and large at 0.80.[29] Statistical calculations were performed using JASP version 0.12.1 based on *R* statistics.

Ethics and patient consent

Written informed consent was obtained from all patients. The study was performed in accordance with the Declaration of Helsinki of the World Medical Association.

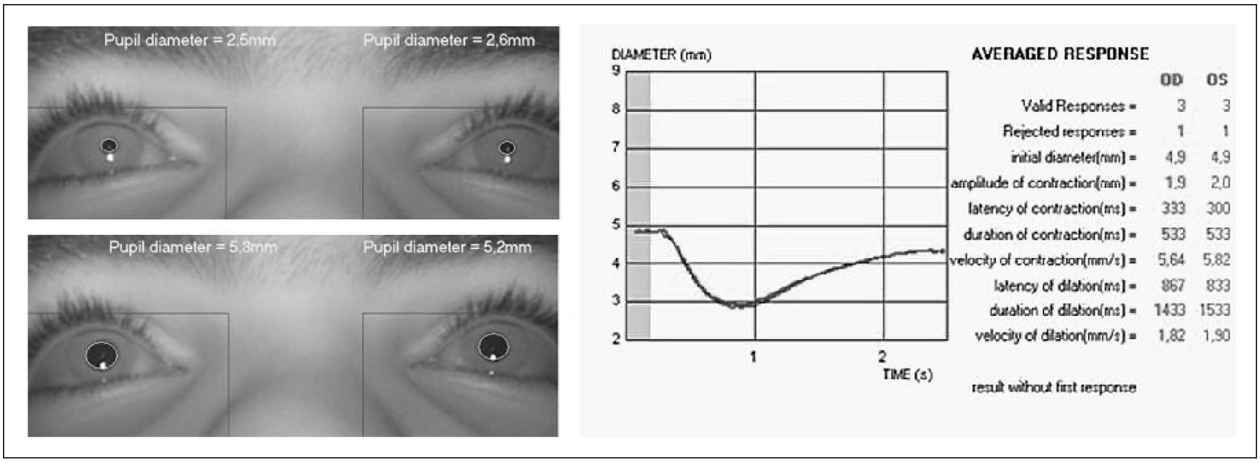

Figure 1. Pupillometry examination case example.

Table 1. Demographic data of the patients.

	ATTRv patients	Healthy controls
Patients, *n*	21	10
Age, years	31.4 ± 6.5	46.8 ± 4.9
Male/female, *n*	8 (38%)/13 (62%)	4 (40%)/6 (60%)
Age of ATTRV30M diagnosis, years	31.4 ± 6.5	–
Age between the diagnosis and treatment, years	3.1 ± 1.3	–
Liver transplantation, *n*	19 (90,5%)	–
Time from liver transplantation, years	12.0 ± 5.7	–
Patients under tafamidis, *n*	2 (9,5%)	–

Results

Demographic data is shown in Table 1. All included eyes had a best-corrected visual acuity of 20/20 or better. No patient presented signs of neuropathy in the neurological examination, namely third cranial nerve or sympathetic dysfunction (ocular movement impairment or ptosis).

Regarding static pupillometry, no significant differences were found in the pupillary diameters under standardized lighting conditions among groups. In dynamic pupillometry, the amplitude of contraction, the velocity of contraction and the velocity of dilation were significantly lower in eyes with scalloped iris, comparing both with the contralateral non-scalloped iris eyes ($p<0.001$ for all) and with eyes from healthy subjects ($p < 0.05$ for all). No differences were found when comparing group 1 and group 0 ($p > 0.100$ for all). Results are further detailed in Table 2 and Figure 2.

Discussion

To our knowledge, this is the first study using automatic pupillometry to objectively evaluate the pupillary light reflex in hATTR patients. Moreover, we used paired eyes from the same patient to better control for interindividual variability, as well as a control group of healthy subjects.

Despite no evident cranial nerve involvement, we found that scalloped irises are associated with changes in the pupillary light reflex, regarding its amplitude and velocity. These changes probably occur due to the accumulation of amyloid in the stroma and muscles of the iris, increasing their stiffness.

We found changes in both dilation and contraction velocities, which are compatible with dysfunction of the dilator and constrictor muscles, respectively. This is corroborated by anatomopathological investigations that revealed amyloid deposits in the iris.[7]

Pupillary abnormalities that have been previously reported in these patients include hyporeactive or unreactive pupils, anisocoria, pupils showing light-near dissociation and bilateral Holmes-Adie pupils.[7,27,30,31] We found no significant differences in static pupillary diameters, but our sample is limited in size and included only patients with no cranial nerve involvement. Anisocoria together with other changes may develop with time. Although, involvement of the autonomic nervous system is well recognized in amyloidosis, cranial nerves are usually spared until late in the evolution of the disease.[6,28] The fact that we found no differences between non-scalloped iris eyes of hATTR patients and control eyes of healthy subjects, supports the mechanical limitation as the cause of the altered pupillary light reflex in scalloped irises (given that in case of

Table 2. Results of static and dynamic pupillometry, comparing group 2 (ATTRv eyes with scalloped pupils) with group 1 (ATTRv eyes without scalloped pupils, second column) and with group 0 (normal eyes of healthy subjects, third column).

	Group 2, mean ± SD	Group 1, Mean ± SD	Paired *t*-test, *p*-value Cohen's *d*	Group 0, mean ± SD	Independ. *t*-test, *p*-value Cohen's *d*
Static					
Scotopic diameter (mm)	5.09 ± 0.76	5.23 ± 0.80	0.053, 0.46	5.48 ± 0.91	0.148, 0.46
Mesopic diameter (mm)	4.06 ± 0.80	4.06 ± 0.74	0.944, 0	4.21 ± 0.72	0.535, 0.20
Low photopic diameter (mm)	3.30 ± 0.77	3.17 ± 0.71	0.249, −0.25	3.43 ± 0.74	0.600, 0.17
High photopic diameter (mm)	2.75 ± 0.64	2.64 ± 0.72	0.140, −0.36	2.85 ± 0.80	0.652, 0.14
Dynamic					
Amplitude of contraction (mm)	1.35 ± 0.46	1.48 ± 0.44	**<0.001, 1.41**	1.71 ± 0.47	**0.017, 0.776**
Latency of contraction (ms)	330.1 ± 71.4	322.2 ± 72.4	0.536, −0.14	316.6 ± 61.4	0.527, −0.200
Duration of contraction (ms)	671.4 ± 112.7	687.2 ± 117.5	0.440, 0.17	673.2 ± 95.8	0.956, 0.02
Velocity of contraction (mm/s)	3.54 ± 1.14	4.22 ± 1.34	**<0.001, 0.99**	4.61 ± 1.33	**0.009, 0.861**
Latency of dilation (ms)	999.9 ± 97.8	1011.2 ± 95.1	0.584, 0.12	990.1 ± 81.0	0.729, −0.109
Duration of dilation (ms)	1454.0 ± 89.7	1431.8 ± 152.8	0.523, -0.14	1478.4 ± 130.7	0.490, 0.22
Velocity of dilation (mm/s)	1.54 ± 0.49	1.74 ± 0.55	**<0.001, 1.04**	1.87 ± 0.58	**0.040, 0.66**

Statistically significant comparisons are highlighted in bold.

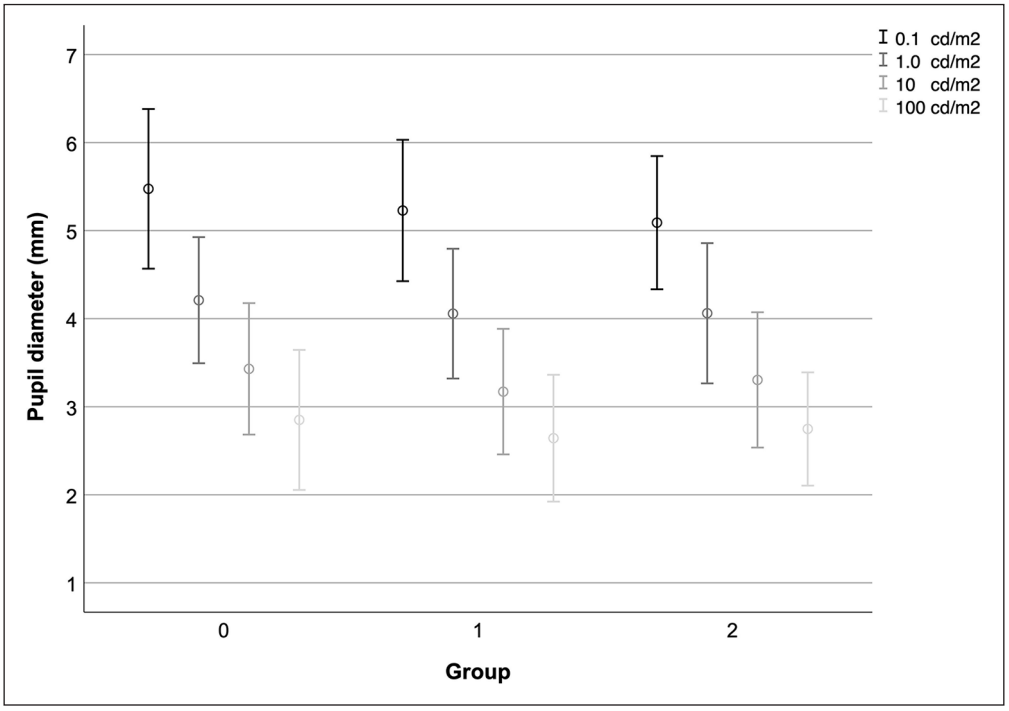

Figure 2. Graph representing static pupillometry diameters by illumination conditions by group.
Marks represent mean and bars represent standard deviation. Illumination units are candle/meter^2 (cd/m^2).

neuropathy, the changes would also be seen in the fellow eye).

On the other side, the presence of scalloped irises is considered a biomarker of intraocular amyloid deposition.[19] Beirão et al.[24,32] reported a strong association between scalloped irises and glaucoma and retinal angiopathy. Pupillometry, as a simple and portable test that may be performed by non-ophthalmologists or technicians, may help to evaluate the presence of amyloid deposits, signaling patients that need an ophthalmological examination, including a screening for glaucoma.

Furthermore, pupillometry is capable of detecting the advent of scalloped iris and consequently it is valuable to evaluate the progression of oculopathy. This may be especially useful while developing new treatments for hATTR (eye-targeted or systemic).

This study has some limitations: histological and objective neurological tests are needed in order to strongly

determine whether these differences result from deposition of amyloid in the iris or from autonomic dysfunction. Besides, a further analysis should determine which pupillometry parameters are directly related to vision-threatening disorders (such as glaucoma and retinal angiopathy).

In conclusion, we found significantly lower amplitude and velocity of contraction and dilation of scalloped iris eyes in hATTR patients, despite no evident neuropathy. There are three main clinical implications after these findings:

(1) The pupillary light reflex is not reliable in hATTR patients to evaluate neurological conditions, like third cranial nerve, sympathetic system or optic nerve dysfunction (evaluated by the presence of a relative afferent pupillary defect).
(2) In the presence of an altered pupillary light reflex in hATTR patients, after investigation of neurological causes, a prompt ophthalmological examination should be considered.
(3) Automatic pupillometry is a simple test that may help to monitor the progression of ocular hATTR.

Authors' note

hATTR - Hereditary transthyretin amyloidosis, denoting in this manuscript the substitution of valine for methionine at position 30.

Author contributions

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published. All authors contributed to the draft of the manuscript, critically reviewed for intellectual content and approved the final version submitted for publication.

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