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# Quantification of relative afferent pupillary defect by an automated pupillometer and its relationship with visual acuity and dimensions of macular lesions in age-related macular degeneration

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**Purpose:** The occurrence of relative afferent pupillary defect (RAPD) secondary to optic nerve diseases and widespread retinal disorders is well established. However, only very few reports of RAPD in macular disorders exist in the literature. In this study, we used automated pupillometer to evaluate RAPD in eyes with macular lesions. **Methods:** It was a prospective cross-sectional study. A total of 82 patients with choroidal neovascular membrane (CNVM) – 65 unilateral and 17 bilateral macular lesions – were enrolled. RAPD was assessed with an automated pupillometer and macular lesions evaluated with optical coherence tomography (OCT). The length of the ellipsoid zone disruption was measured as the longest length of lesion on the horizontal raster scans and the area of macular lesion was measured manually, mapping the affected area of ellipsoid zone on the enface images. **Results:** RAPD scores showed good correlation with the intereye difference in length of maximum ellipsoid zone disruption ($r$-value = 0.84, $P$ value <0.001) and macular lesion area as measured on OCT in all unilateral cases ($r$-value = 0.84, $P$ value <0.001). Best-corrected visual acuity was also found to have a significant correlation with lesion size on the OCT as well as the length of ellipsoid zone disruption in unilateral cases. **Conclusion:** RAPD evaluated with an automated binocular pupillometer is a noninvasive and objective method to assess macular lesions in CNVMs; it shows good correlation with structural lesion dimensions on OCT in unilateral cases. Further longitudinal studies are needed to assess the significance of these findings in disease progression as well as correlation with lesion response to treatment.

**Key words:** Age-related macular degeneration, automated pupillometer, choroidal neovascular membrane, relative afferent pupillary defect

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Relative afferent pupillary defect (RAPD) or Marcus Gunn pupil is a condition in which the response of the two pupils to a flash of light of the same intensity is asymmetrical.[1] It is commonly seen in lesions of the anterior visual pathway that includes retina, optic disc, and optic nerve.[2]

RAPD is usually assessed by the "swinging flashlight" test, first described by Levatin (1959). It involves alternating a flashlight in a regular left-right-left-right eye pattern. In the presence of RAPD, normal eye pupil constricts on illumination while the diseased eye pupil dilates on transferring the light to it.[3,4] The RAPD quantification method using neutral-density filters was later introduced by Thompson *et al*.[5] (1981). The swinging flashlight test is, however, subjected to several external variables and levels of approximation, namely surrounding light, physician experience, and absence of a definite criterion to quantify RAPD.[6–8] Automatic high-resolution infrared pupillometry offers a robust, objective, and accurate alternative to the swinging flashlight test; it has been proposed and tested in different studies – to identify lesions in the optic tract or in the midbrain,[9,10] in normal populations and in patients with various optic neuropathies,[11] in glaucomatous optic neuropathy[12,13], in macular disorders like age-related macular degeneration (ARMD) and disciform macular scars.[2,14]

In our study, we applied a new technology to eyes with macular lesions secondary to ARMD, to investigate the correlation of RAPD scores with visual acuity and dimensions of retinal lesions on optical coherence tomography (OCT). Comparison with standard clinical RAPD evaluation using the swinging flashlight test is also reported and discussed.

## Methods

It was a prospective cross-sectional data collection study conducted from March 2017 to May 2017 in a tertiary eye hospital. We had received approval from the institution's ethics committee for conducting the study. The study followed all the ethical standards of 1964 Declaration of Helsinki and its

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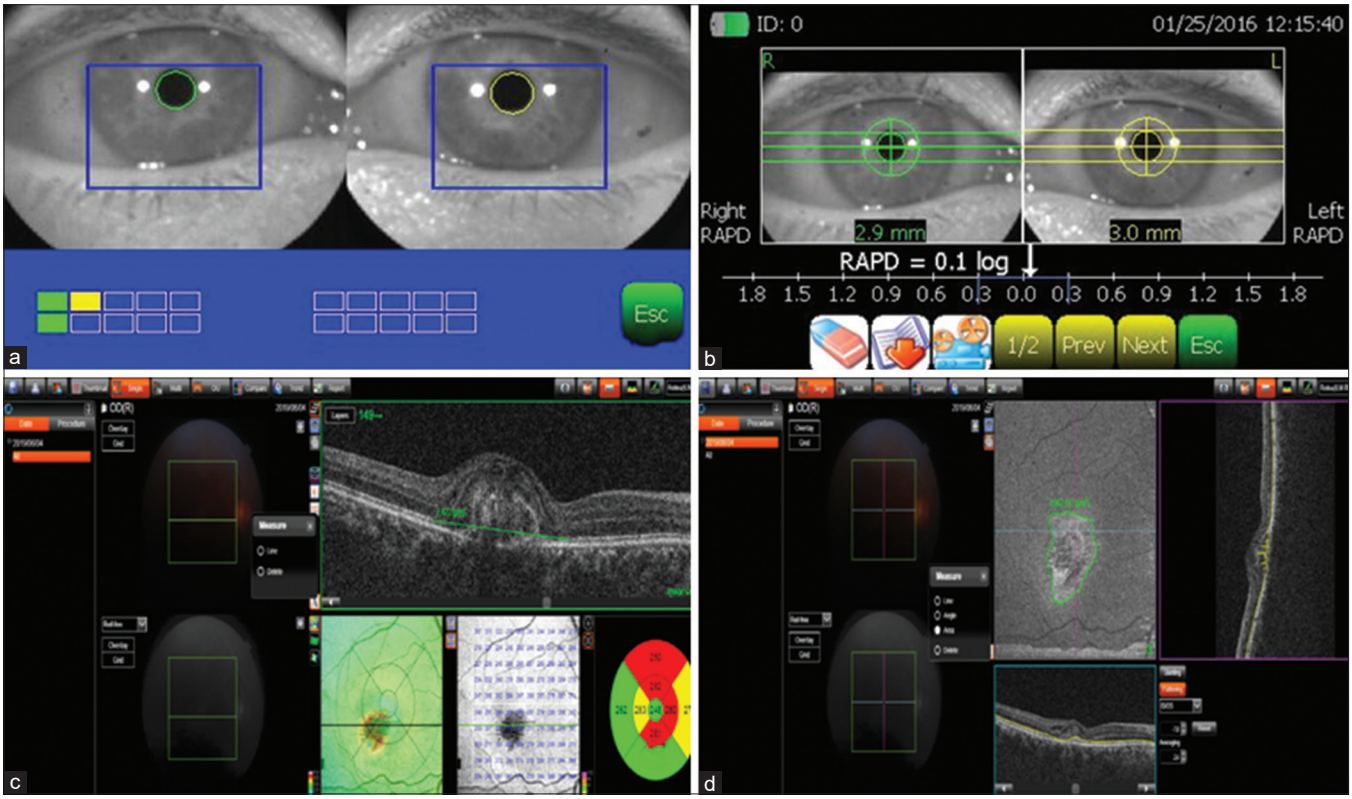


**Figure 1:** Images showing the graphical interface of pupillometer during a measurement; progression is reported by squares being filled in – green implies stimulus successfully delivered, yellow implies stimulus being delivered, and red implies eye blink (a) and results display page (b); images showing length of ellipsoid zone disruption (c) and area of macular lesion measured by manually mapping affected area of ellipsoid zone on the enface images (d)

later amendments. Patients were enrolled based on following inclusion and exclusion criteria, and informed consents were taken.

### Inclusion criteria

- Age ≥30 years
- Bilateral pseudophakia/clear lens/same grade of immature senile cataract
- Retinal pathology: wet ARMD or choroidal neovascular membrane (CNVM).

### Exclusion criteria

- History of any other retinal disease (e.g., diabetic retinopathy)/uveitis
- Any other pupillary abnormalities
- Mature senile and dense nuclear cataracts
- Corneal opacity
- Vitreous opacities
- Optic neuropathy
- On miotics or mydriatics medication
- Diabetic papillopathy.

Ocular examination of the enrolled patients included best-corrected visual acuity (BCVA) measured by Snellen's chart, retinoscopy with subjective and automated refractions, slit-lamp evaluation, intraocular pressure measurement by noncontact tonometer=. Snellen visual acuity was converted to logarithmic minimum angle of resolution for statistical analysis. RAPD assessment was performed by both a swinging flashlight test and automated pupillometry (NeurOptics® RAPiDo™ Binocular Pupillometer) in a dark room.

### Pupillometry

The NeurOptics® RAPiDo™ binocular pupillometer (NeurOptics, Inc., USA) uses infrared technology to give objective and accurate measurements of pupil size, efferent pupillary defect, and RAPD.

The RAPiDo™ algorithm consists of alternating left-right flashes administrated to the subject for 24 s. However, it is extended to 31 s when the patient has multiple blinks [Fig. 1a].

Results are reported in a graphic display [Fig. 1b] that includes a snapshot of the two eyes and their resting pupil sizes [e.g. 2.9 mm, 3.0 mm in Fig. 1b]. RAPD is depicted on a horizontal scale using arrow [e.g. 0.1 log units in Fig. 1b]. The result page also displays a cut-off normative value [e.g. 0.3 log units in Fig. 1b] in the form of blue vertical lines. This value was arrived at by the manufacturers based on unpublished data obtained from healthy volunteers. This value is similar to the values obtained from other similar studies, e.g. study by Wilhem *et al*.[11] and by Pillai *et al*.[13] Measurements were repeated twice by the same examiner to assess intraobserver variability and then averaged for statistical analysis.

Pupillary reactions were first checked manually by a physician in a dark room by swinging flashlight test. It was noted as RAPD present or absent for each eye. Automated RAPD assessment was then done with the pupillometer. All other examinations were performed only after completion of pupil assessment.

### OCT measurement

Pupils were dilated after the assessment of RAPD with 1% tropicamide eye drops. Detailed fundus evaluation was

conducted with slit-lamp biomicroscopy using noncontact +90 D lens. OCT (DRI OCT-1 Model Triton plus, Topcon, Tokyo) of the macula in both eyes was done in all cases. The length of the ellipsoid zone disruption was measured as the longest length of lesion on the horizontal raster scans [Fig. 1c], and the area of macular lesion was measured manually, mapping the affected area of ellipsoid zone on the enface images [Fig. 1d].

### Statistical methods

Categorical variables were presented with frequency and percentage. Mean ± Standard deviation were provided for the continuous variables. Data normality was checked by Shapiro Wilk's test. Spearman's rank correlation was used to test the correlation between continuous skewed variables. We compared the difference between the eyes in terms of lesion area, maximum length of ellipsoid zone disruption, and visual acuity with RAPD scores. ANOVA test was used to compare the different categories of OCT OS, lesions, and BCVA values. Chi-square test used to find the association between categorical variables. Correlations represented with scatter plots. All the analysis performed with STATA software Ver. 14 (Texas, USA).

**Table 1: Correlation for RAPD vs. OCT and BCVA for unilateral and bilateral patients**

| RAPD readings | Unilateral | Bilateral | Total |
|---|---|---|---|
| RAPD Vs OCT ellipsoid zone disruption length | | | |
| *n* | 65 | 17 | 82 |
| Correlation | 0.84 | 0.14 | 0.73 |
| *P* | <0.001* | 0.5846 | <0.001* |
| RAPD Vs OCT macular lesion area | | | |
| *n* | 65 | 17 | 82 |
| Correlation | 0.84 | 0.14 | 0.72 |
| *P* | <0.001* | 0.6044 | <0.001* |
| RAPD Vs BCVA | | | |
| *n* | 65 | 17 | 82 |
| Correlation | 0.83 | 0.53 | 0.79 |
| *P* | <0.001* | 0.0303 | <0.001* |
| OCT ellipsoid zone disruption length Vs BCVA | | | |
| *n* | 65 | 17 | 82 |
| Correlation | 0.82 | 0.42 | 0.79 |
| *P* | <0.001* | 0.0946 | <0.001* |
| OCT macular lesion area VsBCVA | | | |
| *n* | 65 | 17 | 82 |
| Correlation | 0.77 | 0.47 | 0.74 |
| *P* | <0.001* | 0.0561 | <0.001* |

@spearman rank correlation. *Significant correlation (*P*<0.001)

**Table 2: Correlation of RAPD scores with varying lengths of IS-OS disruption and varying areas of macular lesions as measured on OCT**

| OCT ellipsoid zone disruption (microns) | RAPD scores | | | *P** |
|---|---|---|---|---|
| | *n* | Mean (SD) | Min-Max | |
| <2000 | 12 | 0.30 (0.16) | 0.1-0.65 | 0.019 |
| 2000-2999 | 21 | 0.53 (0.4) | 0.1-1.55 | |
| 3000-3999 | 14 | 0.73 (0.45) | 0.3-1.75 | |
| 4000-4999 | 18 | 0.67 (0.42) | 0.1-1.55 | |
| >=5000 | 17 | 0.83 (0.58) | 0.1-1.9 | |
| **OCT lesion area (square mm)** | **RAPD scores** | | | ***P**** |
| | *n* | Mean (SD) | Min-Max | |
| <10.01 | 27 | 0.43 (0.28) | 0.1-1.25 | 0.0035 |
| 10.01-20.0 | 39 | 0.64 (0.42) | 0.1-1.75 | |
| >=20.01 | 16 | 0.93 (0.60) | 0.1-1.9 | |

*ANOVA

## Results

We enrolled a total of 82 patients, which included 47 male and 35 females. Average age was 63.8 ± 12.3 years (range 34–88 years). Sixty-five patients had unilateral macular lesions and 17 had bilateral lesions – a total of 99 eyes with macular lesions were included for OCT scan evaluation and analysis. All the lesions were subfoveal CNVM lesions with classic component. Both active and regressed lesions were included. RAPD was detected on manual evaluation in 31 patients out of a total 82 patients.

RAPD scores measured in automated pupillometer had shown very good correlation with intereye difference in length of maximum ellipsoid zone disruption ($r$–value = 0.84, $P < 0.001$) and macular lesion area as measured on OCT in all unilateral cases ($r$–value = +0.84, $P < 0.001$); correlation had not been found in those with bilateral lesions ($r$-value = 0.14, $P = 0.584$) [Table 1 and Fig. 2a, b]. RAPD scores had also shown significant positive correlation with ellipsoid zone disruption length and macular lesion area grouped into various categories based on the lengths in microns and area in square mm, as shown in Table 2. In our study, many patients even with small lesions on OCT had RAPD scores above the cut-off reference of 0.3 log unit. For example, Table 2 shows 27 pts with lesion area less than 10 $mm^2$ – and a mean RAPD score of 0.43. This is also confirmed by the linear model equation [Fig. 2b] [OCT lesion = 0.68 + 15.49 (RAPD)], where even small lesions of size 5.3 $mm^2$ or above, typically seen in early stages of the disease, already correspond to abnormal values of RAPDs in the range of 0.3–0.4 log units.

Similarly, BCVA was also found to have a significant correlation with OCT lesion size as well as length of ellipsoid zone disruption in all unilateral cases of macular lesion, as shown in Table 1.

A strong and significant correlation had also been found between RAPD scores with BCVA in all cases with unilateral macular pathology ($r = 0.83$, $P < 0.001$), and moderate correlation in bilateral cases [$r$-value = 0.53, $P = 0.03$, Table 1 and Fig. 3].

Finally, when compared to manual assessment and grading of RAPD, automated pupillometer had shown good agreement. RAPD scores from pupillometer had been compared to manual RAPD assessment (RAPD present or absent) for calculation of sensitivity and specificity by using receiver operating characteristics analysis. It had resulted in an area under the curve of 0.94, with 89% sensitivity and 91.7% specificity (parallel study conducted by Pillai *et Al*., 2019).[13]

## Discussion

The parasympathetic afferent pathway of light reflex begins at retina and is mediated by both cones and rods outer retinal photoreceptors and by melanopsin-expressing intrinsically photosensitive inner retinal ganglion cells. The signal is conveyed via the optic nerve to the pretectal nuclei and finally to the oculomotor Edinger–Westphal nucleus (midbrain). Pathologies involving the retina, the ganglion cell layer, and

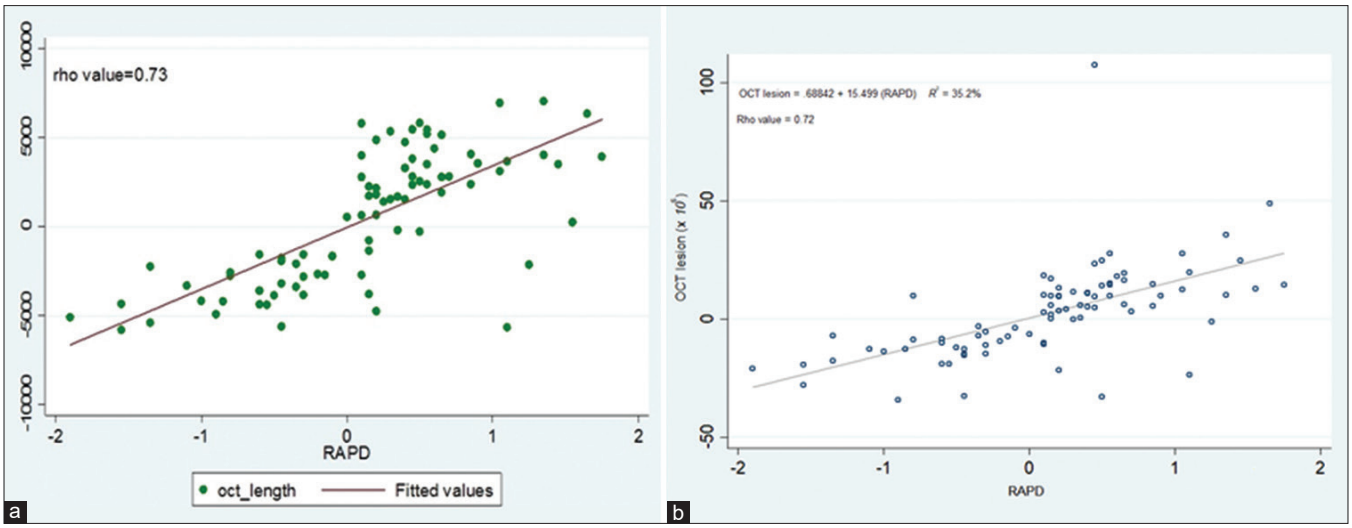


**Figure 2:** Correlation between OCT ellipsoid zone disruption length in millimeters plotted in *y*-axis and RAPD plotted in *x*-axis (a) and correlation between OCT macular lesion area in square mm is plotted in *y*-axis and RAPD plotted in *x*-axis (b)

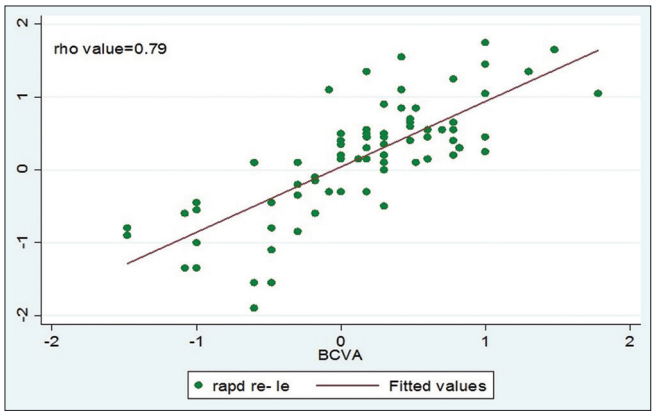


**Figure 3:** Correlation between BCVA (LogMar scale) plotted in *x*-axis and RAPD plotted in *y*-axis

the optic nerve – e.g. optic atrophy, optic neuritis, compressive optic neuropathies, glaucoma, major retinal vessel occlusions, retinal detachment, etc., – all can affect the input signal of the pupil light reflex and its strength.[15] Unilateral or asymmetric diseases generate asymmetric pupil light reflexes that, when compared and related to each other using, for example, the swinging flashlight paradigm, result in a RAPD.

The macula lodges above 50% retinal ganglion cells and provides a significant contribution to the pupillary light responses,[16–18] meaning that photoreceptors at macula are more efficient than the peripheral ones in driving the pupillomotor responses.[14] ARMD and polypoidal choroidal vasculopathy are associated with macular lesions that lead to photoreceptor death and early cone function impairment.[2,19] Focal macular electroretinogram studies in ARMD and submacular bleed have shown an impaired retinal function – with subsequent – improvement following anti-VEGF injections, submacular surgery, or photodynamic therapy.[20–22]

Association between macular degeneration and RAPD was first reported by Newsome *et al.*[14] in eyes with a localized disciform scar in the macula even in the presence of normal dark adaptation – in their study, eyes with RAPD had more frequently larger macular lesions (greater than six disc diameters –62% vs 16%); had longer duration of the lesions (more than 2 years in 74% vs 26%) and worse distant visual acuity (<6/60 in 90% vs 27%) compared to normal eyes. Other pupillary variables, such as the mean constriction amplitude and light reflex latency, are also weakened in eyes with ARMD compared to normal controls and correlated with the greatest linear dimension of the macular lesions.[2] Automated computerized pupillometer has been introduced by Rahman *et al.*[23] in a study where RAPD was correlated with the difference in macular lesion size between two eyes measured with fundus autofluorescence and fundus photography.

Our study here reported provides one more important validation; RAPD is well correlated with the intereye difference in length of maximum ellipsoid zone disruption as well as area of macular lesion size as measured on OCT in unilateral cases. Same correlation was also found with varying lengths of ellipsoid zone disruption and varying areas of macular lesions. In fact, we have seen in our study that even small lesions of size 5.3 mm$^2$ (derived from the linear model equation) or above which are typically seen in the early stages of CNVM do correspond to the abnormal values of RAPD scores in the range of 0.3–0.4 log units. Similarly, BCVA has shown a significant correlation with OCT-based area of lesion and length of ellipsoid zone disruption, and with RAPD readings in unilateral cases. Finally, when compared with manual RAPD assessment, pupillometer results were almost identical – 89% sensitivity and 91.7% specificity (Pillai *et al.*).[13]

Pupillary abnormalities associated with asymmetric macular diseases could be easily overlooked as manual pupil evaluation is usually relegated to clinicians who often only perform a simple, "present" vs. "not present" assessment. Automated pupillometry can be made available and used by all clinical personnel, if it is easy to use and portable as the one used in this study. It would facilitate the initial screening and monitoring of the pupillary pathway. Results can be downloaded and communicated electronically to the physician or simply reviewed in the device screen – its deployment is well suitable for untrained/nontechnical personnel. In a day-to-day clinical practice, it can be used for initial triaging of patients admitted to a facility or telemedicine and mass screening.

Based on this study results, we can advocate that routine evaluation of pupillary reflexes should be conducted prior to fundus evaluation in all patients with macular pathologies. An

afferent pupillary defect may serve as an indicator of degree of impairment of macular function and also help to monitor the subsequent recovery following interventions.[14] Other aspects of the pupil light reflex should also be contemplated. In case of optic nerve pathologies, amplitude of pupil constriction is reduced while latency of onset of pupillary constriction is prolonged. In asymmetrical glaucoma as well as in ARMD, only amplitude seems to be affected.[2,24,25] Difference in the latency can be explained by the suboptimal neuronal fibers conductivity of the diseased optic nerve, and we are planning to further investigate this phenomenon in a follow-up study. This would, however, need a different protocol and was, therefore, beyond the scope of our present study. Macular ERG and its relationship with RAPD are another important and interesting aspect to consider in the future.

## Conclusion

To summarize, RAPD scores using automated binocular pupillometer is a noninvasive, easy to use, and objective method to assess macular lesions in CNVMs; it shows good correlation with structural lesion dimensions on OCT in unilateral cases. Further longitudinal studies are needed to assess the significance of these findings in disease progression as well as correlation with lesion response to treatment.

### Ethics approval

The study was approved by the institute"s ethics committee and study was conducted in accordance with the ethical standards of 1964 Helsinki declaration and its later amendments.

### Consent for publication

Patients signed informed consent regarding publishing their data.

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Nil.

### Conflicts of interest

Dr. Claudio M. Privitera serves as chief scientist at Neuroptics Inc. Other authors in the study have no conflicts of interest.

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