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Reliability of colour perimetry to assess macular pigment optical density in age-related macular degeneration

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Abstract

Background: The aim of this study was to determine the intra-session repeatability and inter-examiner reproducibility of the colour perimetry technique when assessing in vivo macular pigment optical density in age-related macular degeneration patients.

Methods: Age-related macular degeneration patients were classified into four groups: early age-related macular degeneration, intermediate age-related macular degeneration, atrophic age-related macular degeneration and neovascular age-related macular degeneration after undergoing fundus photography (TRC 50DX type IA) and spectral-domain optical coherence tomography analysis (Topcon 3D-2000). Central fixation was confirmed in all patients using the MP-1 microperimeter (Nidek, Padua, Italy). To analyse repeatability, one examiner obtained three consecutive macular pigment optical density measures with MonCV3 device (Metrovision, Perenchies, France). To study agreement between two observers, a second examiner performed another macular pigment optical density measurement in random order. Within-subject standard deviation, coefficient of variation, and intraclass correlation coefficient data were obtained.

Results: Fifty two (32 females and 20 males) consecutive age-related macular degeneration patients having a mean age of 71.5 $\pm$ 8.2 years were recruited. Six had early age-related macular degeneration, 25 had intermediate age-related macular degeneration, 10 had atrophic age-related macular degeneration and 11 had neovascular age-related macular degeneration. For repeatability, coefficient of variation values ranged from 22.3% (neovascular age-related macular degeneration) to 41.0% (atrophic age-related macular degeneration) and intraclass correlation coefficient values from 0.52 (intermediate age-related macular degeneration) to 0.79 (neovascular age-related macular degeneration). For agreement between two examiners, coefficient of variation values ranged from 20.1% (intermediate age-related macular degeneration) to 37.8% (neovascular age-related macular degeneration) and intraclass correlation coefficient values from 0.61 (neovascular age-related macular degeneration) to 0.80 (atrophic age-related macular degeneration).

Conclusion: The reliability (intra-session repeatability and inter-examiner reproducibility) of colour perimetry technique to assess macular pigment optical density in age-related macular degeneration patients is only moderate. Thus, it cannot be recommended to be performed when evaluating and monitoring age-related macular degeneration patients in the daily clinic.

Keywords

Age-related macular degeneration, macular pigment optical density, colour perimetry technique, intra-session repeatability, inter-examiner reproducibility

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Introduction

Age-related macular degeneration (AMD), a degenerative disorder of the central retina with a multifactorial etiology, is the leading cause of visual impairment in adults over 50 years of age in Europe.[1] There has been different clinical classifications of the disease. The more recent one is based on fundus alterations assessed within 2 disc diameters of the fovea. Thus, disease progression criteria from early to late AMD are based on the presence of drusen, pigmentary abnormalities, geographic atrophy or choroidal neovascularization.[2] The use of adequate protocols (i.e. treat–extend–stop) to provide anti-vascular endothelial growth factor (VEGF) therapy makes feasible maintaining or even improving vision in neovascular AMD (nAMD) patients.[3] However, in case of atrophic AMD (aAMD) there is no therapy able to restore the progressive anomaly observed in the retinal pigment epithelium (RPE) or photoreceptors. Thus, patients are recommended to take vitamin supplements, and avoiding smoking or high-alcohol consumption as well as body mass index reduction.[4]

Oxidative stress is associated with both the incidence and the progression of AMD.[5] Initial Age-Related Eye Disease Study (AREDS) trial showed that the oral intake of antioxidants (Vitamins C and E, and beta-carotenes) and zinc produced a reduction of the progression to advanced AMD.[6] Later, the AREDS 2 trial showed that it was worth to replace beta-carotenes form the original AREDS formula, and include lutein and zeaxanthin instead.[7] Carotenoids can be found in orange and yellow fruits and green leafy vegetables, and lutein and zeaxanthin are one of the most frequently eaten carotenoids. Besides, macular pigment (MP) is made up of the three carotenoids lutein, zeaxanthin, and meso-zeaxanthin (synthesized from lutein), and they are highly concentrated on the macular region (macula lutea), decreasing its concentration rapidly with eccentricity.[8] It is thought that MP has a protective role from the damaging effects of free radicals produced by blue light, and it could also avoid development and progression of AMD. Although low dietary and blood carotenoids were assumed to be modifiable risk factors for developing AMD, the protective effect of MP in the retina remains unclear. Consequently, in vivo evaluation of MP has become an important matter in the assessment and management of AMD patients.

In vivo measurement of MP optical density (MPOD) can be performed using different techniques that could be based on physical methods like fundus autofluorescence,[9] fluorescence lifetime imaging ophthalmoscopy,[10] fundus reflectometry,[11] resonance Raman spectroscopy[12] and visual evoked potentials,[13] or psychophysical ones like heterochromatic flicker photometry (HFP)[14] and minimum motion photometry.[15,16] Another psychophysical technique to evaluate MP is colour perimetry.[17] It is a simple non-invasive method that compares colour sensitivity outcomes between two wavelengths (blue and red) differently absorbed by MP. Nonetheless, the reliability of the measurements obtained by any ophthalmic instrument should be determined to establish its clinical value. Consequently, the aim of the present study was to estimate the intra-session repeatability and interobserver reproducibility of the colour perimetry technique to assess MPOD in AMD patients.

Materials and methods

This prospective observational study was approved by the East Valladolid Area Ethics Committee (Valladolid, Spain) and complied with the tenets of the Declaration of Helsinki. All candidates received detailed information about the nature of the investigation, and all provided their written consent.

Participants

The study recruited consecutive Caucasian patients with a diagnosis of AMD. Inclusion criteria for all participants were the following: age $\geqslant$55 years and distance best-corrected visual acuity (BCVA) $\geqslant$20/40. Exclusion criteria included other ocular disease different from AMD (especially cataract), any other physical or cognitive anomaly that could alter the performance of the clinical tests, and following any treatment that could affect visual field.

To assure that AMD patients had central fixation, all patients underwent fixation analysis using the MP-1 microperimeter (Nidek, Padua, Italy). Patients were requested to stare at a white fixation cross (3 degrees height) presented on a dark background. They were allowed to use their preferred retinal locus. The procedure lasted 30 s. The MP-1 device is able to monitor eye position by tracking a retinal landmark at 25 Hz. Central fixation was defined as having more than 50% of the preferred fixation points located within 2 degrees of the fovea.

At the screening visit, inclusion and exclusion criteria were checked. Distance BCVA was determined using the Early Treatment Diabetic Retinopathy Study chart (Lighthouse, Long Island, NY). Later, fixation analysis was performed with MP-1 device. Then, fundus colour and autofluorescence images were obtained (TRC 50DX type IA; Topcon Europe Medical B.V., The Netherlands) within an area of 2 optic disc diameters centred in the fovea by using the device software (Topcon IMAGEnet i-base versión 3.14.4). A spectral-domain optical coherence tomography (SD-OCT) was also obtained (Topcon 3D-2000; Topcon Europe Medical B.V) with the 3D Macula protocol. It consists of a raster scan composed of 256 $\times$ 256 (vertical $\times$ horizontal) axial scans covering an area of 6 $\times$

6 mm in the macular region. Consecutive volunteers were included in the following groups depending on the screening outcomes:[2]

- Early AMD (eAMD): Presence of drusen >63 μm and ⩽125 μm, without RPE alterations in the macular area.
- Intermediate AMD (iAMD): Presence of drusen >125 μm and/or RPE alterations in the macular area.
- Advanced AMD subdivided into two groups:
  - aAMD: Presence of hypopigmented area of 175 μm with visible choroidal vessels within the studied area. Only patients with foveal sparing were included.
  - nAMD: History of non-subfoveal choroidal neovascularization secondary to AMD. Patients should show absence of any sign of activity (presence of subretinal fluid, or increase in thickening of the retina, or blood, or decrease in vision) at the time of inclussion.

Colour perimetry technique

Colour perimetry (MonCV3; Metrovision, Perenchies, France) is a psychophysical technique that makes use of the spectral absorption properties and retinal location of MP in order to assess MPOD.[17] The technique is based on the comparison of the thresholds obtained for blue and red light perception. Thresholds are measured in decibels (dB) and are estimated following bracketing strategies. A staircase 4-2-2-2 full-threshold strategy was used for the present study. The measurement procedure is based on the estimation of luminance differential thresholds for a blue and red stimulus. MP selectively absorbs blue incident light, with maximum absorption around 460 nm and no absorption above 530 nm.[18] Therefore, MPOD can be estimated as the difference in blue and red stimuli thresholds observed for macular and para-macular zones. In our study, stimuli were projected to the macula and to six different eccentric (10 degrees) locations, where MP is assumed to be negligible in comparison with the macular area. The stimulus had a Goldmann size III and was presented on a white background of 10 cd/m^2. Outcomes are provided in units of decibels.

When macular sensitivity was evaluated, participants should stare at the centre of an empty black circle over a white background, and blue and red stimuli were presented in the middle. When para-macular sensitivity was assessed, a central dot was showed, and blue and red stimuli were presented at paracentral locations (10 degrees). Participants performed the colour perimetry test for the first time during the screening visit; once inclusion and exclusion criteria were checked (and after signing the inform consent). This first test was a trial run and it was discarded, because we wanted to avoid learning effect and also allow familiarity with equipment and test.

Statistical analysis

To estimate the intra-session repeatability, it is recommended to perform independent test using the same method on the same patient with the shortest time possible. Thus, participants underwent three consecutive MPOD evaluations performed by examiner 1. To estimate the intra-session repeatability, the within-subject standard deviation (Sw) of three consecutive measurements was calculated to obtain the intra-session coefficient of variation (CVw), which is defined as the ratio of the Sw over the mean (expressed as a percentage).[19] In addition, the intra-session reliability was also estimated using the intraclass correlation coefficient (ICC),[20] which was obtained after performing a one-way analysis of variance with repeated measures.

To estimate the agreement between examiners (inter-examiner reproducibility), a second examiner performed only one MPOD evaluation. The order of the procedures performed by each examiner was random to avoid bias. Also, MPOD assessment was performed within the shortest time to prevent fatigue bias. The first of the three MPOD evaluations performed by the first examiner was the one computed to establish agreement between examiners. The inter-examiner reproducibility was estimated by calculating first the inter-examiner Sw, and then, the inter-examiner CVw.[19] In addition, the interobserver ICC was also estimated. The paired t test was used to establish whether there was a significant systematic bias between examiners.

Data from the prospectively completed forms were entered into a database, and statistical calculations were performed by a statistician (I.F.), using the R statistical package version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Data distribution was evaluated using the Shapiro–Wilk test. The mean and the SD were calculated for normally distributed data. Spearman rank correlation coefficient was used to measure the association between the mean MPOD values and the SDw of the three measurements (intra-session repeatability) or two measurements (interobserver reproducibility) to confirm the assumption that the amplitude of variation was unrelated to the MPOD magnitude before proceeding with reliability analyses. An analysis of variance was performed to assess difference among the three MPOD measures obtained for each group. Two-tailed $p \leqslant 0.05$ was considered significant for all statistical tests.

Results

A total of 52 consecutive AMD patients (32 females and 20 males) were recruited. Six were included in eAMD

Table 1. MPOD intra-session repeatability values as measured with the colour perimetry technique.

Group	MPOD value (dB)	Sw (dB)			CVw (%)		
	Mean ± SD	Mean	95% CI		Mean	95% CI	
eAMD	5.73 ± 2.50	1.33	0.52	2.12	27.00	10.72	43.26
iAMD	5.82 ± 2.19	1.57	1.16	1.97	29.46	21.35	37.56
aAMD	6.14 ± 3.31	1.73	0.81	2.64	41.03	15.92	66.12
nAMD	6.97 ± 3.06	1.35	0.91	1.79	22.29	12.31	32.27

MPOD: macular pigment optical density; Sw: within-subject standard deviation; CVw: within-subject coefficient of variation; CI: confidence interval; AMD: age-related macular degeneration; eAMD: early AMD; iAMD: intermediate AMD; aAMD: atrophic AMD; nAMD: neovascular AMD.

Table 2. MPOD inter-examiner reproducibility values as measured with the colour perimetry technique.

Group	MPOD value (dB)	Sw (dB)			CVw (%)		
	Mean ± SD	Mean	95% CI		Mean	95% CI	
eAMD	6.47 ± 2.16	1.06	0.34	1.77	20.64	2.62	38.65
iAMD	5.73 ± 2.19	1.03	0.70	1.34	20.12	13.22	27.02
aAMD	6.08 ± 3.28	1.12	0.42	1.80	32.99	2.22	63.74
nAMD	6.82 ± 3.51	1.76	0.82	2.68	37.77	8.87	66.66

MPOD: macular pigment optical density; Sw: within-subject standard deviation; CVw: within-subject coefficient of variation; CI: confidence interval; AMD: age-related macular degeneration; eAMD: early AMD; iAMD: intermediate AMD; aAMD: atrophic AMD; nAMD: neovascular AMD.

group, 25 in the iAMD group, 10 in the aAMD group and 11 in the nAMD group. There were three pseudophakic patients in the aAMD group and three in the nAMD group. All of them were previously implanted with the same intraocular lens (AcrySof Natural; Alcon Laboratories Inc., Fort Worth, Texas, USA). The mean age of the eAMD, iAMD, aAMD and nAMD group was 67.8 ± 3.9, 73.3 ± 8.4, 70.0 ± 3.1 and 77.4 ± 8.7 years, respectively. There were not significant ($p = 0.07$) differences in age among the groups included.

Intra-session repeatability

Table 1 shows the global mean, Sw and CVw observed in AMD groups for the intra-session repeatability estimations. There were not significant differences ($p = 0.66$) among the mean MPOD values obtained for each study group. ICC value observed for the eAMD group was 0.72 (95% confidence interval (CI): 0.28–1.00); for the iAMD group, 0.52 (95% CI: 0.28–0.75); for the aAMD group, 0.68 (95% CI: 0.35–1.00); and for the nAMD group, 0.79 (95% CI: 0.58–1.00).

Inter-examiner reproducibility

Mean differences in MPOD values between both examiners (examiner 1 and examiner 2) were not significant for the iAMD group (0.18 dB (95% CI: –0.58–0.93; $p = 0.63$)), for the aAMD group (0.11 dB (95% CI: –1.42–1.65; $p = 0.87$)), nor for the nAMD group (0.31 dB (95% CI: –1.87–2.48; $p = 0.76$)). However, significant ($p = 0.01$) mean differences were observed for the eAMD group (–1.50 dB (95% CI: –2.50, –0.49)).

Table 2 shows the global mean, Sw and CVw observed in AMD groups for the interobserver reproducibility estimations. ICC values observed for the eAMD group, 0.72 (95% CI: 0.18–1.00); for the iAMD group, 0.66 (95% CI: 0.42–0.89); for the aAMD group, 0.80 (95% CI: 0.54–1.00); and for the nAMD group, 0.61 (95% CI: 0.17–1.00).

Discussion

MP carotenoids, lutein, zeaxanthin and meso-zeaxanthin have been assigned a putative protective role for AMD based on their ability to become absorbers of harmful light and antioxidants reacting with reactive oxygen species.[21] Several studies have showed in different populations that diet plays an important role in the progression of AMD, because a diet rich in lutein or zeaxanthin decreases the risk of AMD.[22–24] Consequently, assessing in vivo MP has become an important issue when dealing with AMD patients. Colour perimetry is a psychophysical technique able to indirectly assess MPOD, and as any other ophthalmic evaluation techniques, should provide reliable measurements to avoid misleading when counselling AMD patients.

In our study, we evaluated the intra-session repeatability of the colour perimetry technique in AMD patients, and we found CVw values above 22% for all the AMD groups evaluated (Table 1). We did not find differences in mean MPOD values among the three consecutive measurements

performed; however, colour perimetry outcomes can vary above 22% in consecutive measurements, thus the technique cannot be considered adequate for clinical purposes. Regarding intra-session repeatability ICC values, all of them were below 0.8 (ICC for nAMD = 0.79). The absence of a high ICC value (>0.90) indicates that most of the variability observed for the three MPOD measures is due to differences within the same AMD patient, instead of differences among all AMD patients recruited. Therefore, these repeatability outcomes show that colour perimetry technique provides only moderate agreement among MPOD measurements obtained during the same session.

We decided to perform an inter-examiner reproducibility study because it has been already highlighted the importance of the data interpretation for MPOD results, at least when performing the HFP technique.[25] In case of colour perimetry technique, it can be initially thought that examiner might not play such an important role; however, it is a psychophysical test like conventional automated perimetry. And for this later technique, it has been reported that threshold is affected by the way examiner delivers instructions, because conservative (vs liberal) instructions can cause patients to be more reluctant to respond.[26] Consequently, it was worth to assess the agreement between two different examiners when performing the colour perimetry technique. Similarly to the outcomes observed for intra-session repeatability analysis, inter-examiner reproducibility measurements yielded CVw values above 20% (Table 2) and ICC values ⩽0.80. Inter-examiner reliability outcomes were not poorer than intra-examiner ones; however, they are not good enough to recommend colour perimetry as a consistent tool to assess MPOD.

We were able to find in the literature only another study using colour perimetry to assess MPOD. Demirel et al.[27] evaluated the inter-session reproducibility of colour perimetry after assessing healthy volunteers during three visits in three consecutive days. These authors obtained a wide range of ICC results (0.48–0.82) depending on the visits selected for the inter-session reproducibility estimation. These ICC values are within the range that we obtained in different AMD groups. These findings further support that the reliability of colour perimetry to assess MPOD is low.

The reliability of other psychophysical techniques to assess MPOD has been also estimated, despite different statistical variables have been used to estimate it. Loughman et al.[28] obtained MPOD values during three different sessions using the HFP technique in healthy subjects. Based on the data they reported, their lowest inter-session reproducibility CVw value was around 28% for the MPS 9000 device (Tinsley Precision Instruments Ltd., Croyden, UK), and close to 14% for the Macular Densitometer device (Macular Metrics II, Rehoboth, MA, USA). Likewise, De Kinkelder et al.[29] obtained MPOD values using two different devices (Macuscope; Macuvision Europe Ltd., Lapworth, UK, and QuantifEye; MPS 9000 series), and estimated relative differences (defined as the differences of two values divided by their mean value) between two consecutive measurements. They obtained values of 32.2% and 18.1% for each instrument, respectively. Taking into account that the variability of MPOD as measured with the HFP technique was high, Howells et al.[25] proposed a new improved protocol for the MPS 9000 device. After evaluating healthy volunteers with the new protocol, they obtained better CVw values for intra-session and inter-session tests (around 19% and 12% based on the data reported, respectively).

Regarding the inter-rater agreement of the HFP technique, Bartlett et al.[30] evaluated young normally sighted volunteers who underwent HFP assessment using the MPS 9000 instrument (also known as QuantifEye). They evaluated volunteers using two different observers during the same session (same day) on two occasions separated by 1 week. Based on the data that they reported, the CVw they obtained was likely to be >35%. However, this study was performed prior to the upgrade of the measurement procedure later published by Howells et al.[25]

Random error estimated in the present study prevents from advising the use of colour perimetry to monitor MP in AMD patients. We did not find significant differences between the three consecutive tests that we performed for estimating the intra-session repeatability. Therefore, it seems that there was not either fatigue or learning effect in our AMD patients. The source of the random error that we found might be inherited from the psychophysical nature of the procedure. We performed a fixation test analysis with a microperimeter to ensure central fixation in our AMD patients, so that participants could stare at the fixation targets presented during the procedure. However, it does ensure that patient keeps their gaze properly aligned during the whole test. The device that we used to perform the colour perimetry technique has got an eye-tracker, so that patient could be monitored throughout the whole procedure, and instructions could be provided in case exists excessive eye movement. Besides, the colour perimetry technique requires also to project stimuli to paracentral areas (10 degrees eccentricity), and these zones might be affected because of the AMD disease. Consequently, reliability outcomes observed in our study are expected to be reduced in comparison with other studies that reported reliability data from other MPOD techniques recruiting only healthy young adult volunteers.

As previously mentioned, in vivo MPOD measures can be also obtained using physical methods, and fundus reflectometry might be the most common. Thus, reliability studies have been also performed to assess its clinical validity. Dragostinoff et al.[11] reported a CVw value of 6.2% and 8.0% in the healthy and AMD patients, respectively, after performing 5 evaluations in 5 consecutive days using a custom-built densitometer. Moreover, Creuzot-Garcher et al.[31] reported good ICC values (>0.80) when measuring

MPOD with a commercial reflectometry technique (Visucam 200; Carl Zeiss Meditec AG, Jena, Germany), as long as the same examiner performed always the intra- and inter-session tests. Consequently, based on the reliability data reported, it seems that physical (objective) techniques should be used to evaluate MPOD instead of psychophysical (subjective) ones, to ensure more consistent data.

One limitation of the present study is the sample size. Taking into account the large prevalence of AMD in Occidental countries, we cannot ensure that our AMD sample fully represents the whole population of Caucasian AMD patients. Nonetheless, our aim was to estimate the reliability of the colour perimetry technique to measure MPOD, and we recruited enough diverse AMD patients to provide valuable results for the daily clinic. Another limitation is that crystalline lens transmission of blue light decreases with age;[32] thus the relationship between blue and red transmission can be altered in elder patients, and it might have affected our outcomes. Nonetheless, AMD patients having cataract, even mild, were not recruited to avoid as much as possible this limitation. Another limitation is that the device used to test colour perimetry did not allow for fundus-tracking in contrast to other systems.[33] Nonetheless, the equipment had an automated ocular fixation control, so that at least eye movements could be monitored throughout the test.

In conclusion, the intra-session repeatability and inter-examiner reproducibility of colour perimetry technique to evaluate MPOD in AMD patients is not good enough to advice the use of this psychophysical technique in the clinical practice. Literature shows that objective techniques can provide better reliability MPOD outcomes than subjective ones. Thus, instruments based on objective techniques should be recommended for clinical purposes when counselling AMD patients in their early-intermediate stages of the disease, taking into account that adequate diet supplementation produces short-term[34] and long-term[6,7] MP improvements.

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