Macular Pigment Optical Density in a South Indian Population

Rajiv Raman,1 Rajni Rajan,2 Sayantan Biswas,2 Kulothungan Vaitheeswaran,3 and Tarun Sharma1

PURPOSE. To estimate the normal value of macular pigment optical density (MPOD) in an adult south Indian sample.

METHODS. Three hundred eyes of 161 healthy volunteers (30 men and 30 women in each of the age groups of 20–29, 30–39, 40–49, 50–59, and ≥60 years) underwent MPOD measurement with a macular densitometer. Thirty-two eyes were also checked for intersession variability.

RESULTS. The mean MPODs in the Indian sample were 0.64 ± 0.23 log unit at 0.25° eccentricity, 0.50 ± 0.21 log unit at 0.5°, 0.37 ± 0.19 log unit at 1.0°, and 0.21 ± 0.16 log unit at 1.75°.

Of all the foveal eccentricities, the MPOD showed an increase from 20 to 29 to 30 to 39 years of age and thereby showed a decrease with age. The men aged 40 to 49 years had significantly higher MPOD than did the women (0.75 vs. 0.62 log unit, \(P = 0.039\)), and the women aged 50 to 59 years had higher MPOD than did the men (0.71 vs. 0.57 log unit, \(P = 0.019\)). There was no significant intersexes or interocular variation.

CONCLUSIONS. This study establishes the MPOD normogram in an adult Indian sample. (Invest Ophthalmol Vis Sci. 2011;52:7910–7916) DOI:10.1167/iovs.11-7636

Macular pigment consists of two hydroxy carotenoids: lutein and zeaxanthin.1,2 Visually, macular pigment presents as a yellow coloration concentrated in the fovea. Fundus reflectance and autofluorescence maps show ring patterns in the distribution of the macular pigment.3 Macular carotenoids reach their highest levels in the axons of the photoreceptors and inner plexiform layers of the retina.2–5 It has been proposed that macular carotenoids are related to visual performance in both normal subjects and those with ocular pathologies.6–13 Epidemiologic studies have shown a controversial relationship between age-related macular degeneration (AMD) and the macular pigment optical density (MPOD). Although a few studies have shown an inverse relationship between AMD and MPOD11–14 most failed to prove any relationship.15–17

There are ethnic differences in macular pigment distribution. It is reported to be higher in African (0.59 ± 0.14 density units [DU]) than in white (0.36 ± 0.13 DU) non-Hispanics.16 Tang et al.19 estimated the average MPOD in Asian Chinese to be 0.48 ± 0.23, which was higher than in the white non-Hispanics but lower than in the Africans. Age and MPOD have a controversial relationship, with several studies, with various sample sizes and different techniques reporting different relationships between the two.20 Lam et al.21 showed a decline in macular pigment with age and significant sex differences in the middle age groups. Most of the studies are not comparable because of the difference in measuring techniques used, including heterochromatic flicker photometry (HFP), high-performance liquid chromatography, Raman resonance spectroscopy, and auto fluorescence.20

The relationship between sex and MPOD is equally controversial, with a few studies finding males having 13% to 30% higher MPOD than females,22–24 whereas other found no effect of a subject’s sex on MPOD.11,19,25–28

Reports on macular pigment density in an adult urban Indian population are not available. This study was conducted to fill this gap.

MATERIALS AND METHODS

Normative data were collected from 300 eyes of 161 healthy volunteers of south Indian origin working in our institute and/or their family members between April 2008 and March 2009. From 322 eyes of 161 subjects, 22 eyes with a history of cataract surgery were excluded in the final analysis. The sample was divided equally among the age groups of 20 to 29, 30 to 39, 40 to 49, 50 to 59, and ≥60 years; with each group containing 30 men and 30 women. The study was approved by the Institutional Review Board, and a written informed consent was obtained from the subjects per the Declaration of Helsinki. Demographic data including age, sex, education, and the anthropometric measurements height, weight, waist, and hip were collected. Subjects included in the study had corrected visual acuity of 20/20 or better. Those with any ocular pathology, history of ocular surgery, systemic illness such as diabetes mellitus, hypertension, a family history of AMD, past/present smoking, or a regular intake of carotenoids/vitamins/antioxidants were excluded. A comprehensive ocular examination was conducted, followed by the assessment of MPOD with a macular densitometer (Macular Metrics Corp., Rehoboth, DE). All the subjects were naive about performing psychophysical tasks.

Macular densitometer is based on the principle of HFP. The basic measurement procedure involves presenting a small test stimulus that alternates between a measuring wavelength (460 nm) which is absorbed by macular pigments and a reference wavelength not absorbed by the pigments (540 nm). This stimulus is presented in the fovea. To the subject, this alternating stimulus appears as a small flickering light. The subject is given control of the intensity of the measuring light and the task of adjusting it to minimize the flicker. Flicker could be eliminated (null zone) if the luminance of the 460-nm light was increased to match that of the 540-nm light. This increase in luminance was related to the optical density of the macular pigment (i.e., if there was a larger amount of pigment present, the absorption of the 460-nm light would be greater and its luminance would have to be increased to match that of the 540-nm light). The amount of 460-nm light required to produce

From 1Shri Bhagwan Mahavir Vitreoretinal Services, Tamil Nadu, India; the 2Elite School of Optometry, Tamil Nadu, India; and the 3Department of Preventive Ophthalmology (Epidemiology and Biostatistics), Tamil Nadu, India.

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Corresponding author:. Rajiv Raman, Shri Bhagwan Mahavir Vitreoretinal Services, Sankara Nethralaya, 18, College Road, Chennai, 600 006, Tamil Nadu, India; rajivpgraman@gmail.com.
this null zone provides a measure of the MPOD at the retinal location of the test light. The alternation frequency must be optimized for each subject and the test stimuli used at each locus (see below).

Each eye was tested randomly to minimize any potential order effect. Figure 1 shows the targets used for measuring MPOD; for 0.25° foveal eccentricity it was a solid disc of 15 min arc radius; for 0.50°, a solid disc of 0.5° arc radius; for 1.00°, an annulus with an inner radius of 50 min arc and an outer radius of 70 min arc; and for 1.75° an annulus with an inner radius of 90 min arc and an outer radius of 120 min arc. For the parafoveal measurement, the subject was asked to focus on a red light located precisely at 7° from central fixation. Subjects requiring distance refractive error correction were provided with the correction in trial frames or were allowed to wear their spectacles.

A small black dot was present at the center of the solid disks as a fixation aid and a fixation target of a 5-min arc radius was centered within each annulus for the same. The test stimulus was superimposed on a 6.00°, 1.5 log Td, 470-nm circular blue background for the foveal measurement of the MPOD. Target 1 for 25° foveal eccentricity: a solid disc of 15 min arc radius. Target 2 for 0.50° foveal eccentricity: a solid disc of 0.5° arc radius. Target 3 for 1.00° foveal eccentricity: an annulus with an inner radius of 50 min arc and an outer radius of 70 min arc. Target 4 for 1.75° foveal eccentricity: an annulus with an inner radius of 90 min arc and an outer radius of 120 min arc. Target 5 for 7° temporal eccentricity: test target of 1° radius.

The starting flicker frequency was set at 10 to 11 Hz. If the subject was unable to find a zone of no/minimal flicker, this frequency was increased until a zone of no/minimal flicker could be identified. If the null zone was wide, the flicker frequency was reduced. The flicker frequency was adjusted until the subject found a narrow null zone. For participants who had difficulty adjusting the flicker on their own, we performed the task on their behalf, instructing the subject to notify us immediately on cessation of the flicker sensation. A minimum of three readings (radiance measurements of the 460-nm light, which provides a null zone) were taken at each eccentricity.

Subjects were constantly instructed to blink several times and to continue adjusting the knob until the blinking no longer allowed the sensation of flickering in the test targets to resume. Readings were deemed reliable and included in the study only if the standard deviation of the readings (radiance measurements of the 460-nm light which provides null zone) did not exceed 20%.

MPOD was measured using the formula, $\text{MPOD} = \frac{-\log_{10}(R_f/R_p)}{p}$, where $R_f$ is the radiance of the 460-nm light needed for the null zone at the foveal location being measured, and $R_p$ is the radiance of a null zone at the reference location in the parafovea, where MPOD is negligible. (MPOD is derived by subtracting the log foveal sensitivity from the log parafoveal sensitivity, measured at the 7° parafoveal reference point). It was calculated using the manufacturer’s software provided by the Macular Metrics Corp., which fits an exponential function to the data and plots the spatial profile of the subject’s MPOD.

MPOD measurements were repeated in 32 eyes to assess the intersession variability of the readings. The duration between the first session and the repeat session ranged from 1 to 4 weeks depending on the compliance of the subjects.

The normality distribution was checked for all quantitative variables. One-way ANOVA was used to compare between eccentricity (four levels) as a dependent variable and age group (1 decade for each level) as a factor. Two-way ANOVA was used, with eccentricity as the dependent variable and sex as a factor by age group. Multiple comparisons were performed with Bonferroni correction. Post hoc power was calculated by G Power 3.0. Test-retest repeatability was assessed with the intraclass correlation coefficient (Cronbach’s $\alpha$) and the agreement between the MPOD measurements obtained at first and repeat sessions were assessed with Bland-Altman plots. $P \leq 0.05$ was considered significant (SPSS 14.0; SPSS Inc., Chicago, IL).

**RESULTS**

Three hundred eyes of 161 volunteers who fulfilled the inclusion and exclusion criteria were recruited for the study. Figure 2 shows the frequency distribution of the MPOD values at 0.25°, 0.50°, 1.00°, and 1.75° foveal eccentricities. The mean MPOD was 0.64 ± 0.23 log unit (range, 0.08–1.29) at 0.25° foveal eccentricity, 0.50 ± 0.21 log unit (0.00 to 1.11) at 0.50°, 0.37 ± 0.19 log unit (−0.15 to 0.98) at 1.00°, and 0.21 ± 0.16 log unit (−0.17 to 0.82) at 1.75°. The mean time ± SD taken to measure MPOD at all four foveal eccentricities monocularly was 18 ± 8 minutes (range, 6–51 minutes).

Figure 3 shows the mean MPOD at each eccentricity for the different age groups. In Figure 3A, the mean MPOD at 0.25° foveal eccentricity shows an increase from 20 to 29 years (0.35 ± 0.16 log units) to 30 to 39 years (0.72 ± 0.22 log units). Thereafter, MPOD declined in age groups 40 to 49, 50 to 59, and ≥60 years (0.69, 0.64, and 0.51, respectively). Figure 3B shows the MPOD at 0.50° foveal eccentricity to be increased from 20 to 29 years (0.51 ± 0.16 log units) to 30 to 39 years (0.55 ± 0.23 log units). Thereafter, MPOD declined in the age groups 40 to 49, 50 to 59, and ≥60 years (0.52, 0.50, and 0.41, respectively). Similarly, the MPOD at 1.00° foveal eccentricity (Fig. 3C) shows an increase from 20 to 29 (0.39 ± 0.16 log units) to 30 to 39 (0.42 ± 0.20 log units) years. Thereafter, MPOD declined in age groups 40 to 49, 50 to 59, and ≥60 years (0.40, 0.36, and 0.27, respectively). Although a similar trend was seen at 1.75° foveal eccentricity (Fig. 3D), it was not statistically significant ($P = 0.401$).
The mean MPOD values in men and women at 0.25°, 0.50°, 1.00°, and 1.75° foveal eccentricities are summarized in Table 1. There were no differences between the sexes in mean MPOD until age 40 years. Between 40 to 49 years, the men had higher MPOD than the women at 0.25° (0.75 ± 0.28 in men vs. 0.62 ± 0.18 log unit in women; P = 0.039). Between 50 and 59 years of age, women had higher MPODs than men at the 0.25°, 0.50°, and 1.00° foveal eccentricities (0.57 vs. 0.71 at 0.25°; P = 0.019, 0.45 vs. 0.55 at 0.50°; P = 0.041, and 0.30 vs. 0.42 at 1.00°; P = 0.017, respectively). After 60 years, there again was no difference in mean MPOD between the sexes.

The mean interocular differences were approximately 0.04 ± 0.15, 0.03 ± 0.14, 0.03 ± 0.14, and 0.02 ± 0.12 at 0.25°, 0.50°, 1.00° and 1.75° foveal eccentricities, respectively; none was statistically significant. Figure 4 shows the Bland-Altman plots obtained at the first and repeat sessions. The mean difference (± SD) in MPOD at 0.25° between the first and repeat sessions was −0.01 (±0.11), with a 95% confidence interval for this mean difference of 0.21 to −0.24. The mean difference (± SD) in MPOD at 0.50° between the first and repeat session was 0.0 (±0.12) with a 95% confidence interval for this mean difference of 0.25 to −0.26. The mean difference (± SD) in MPOD at 1.75° between the first and repeat session was 0.0 (±0.09) with a 95% confidence interval for this mean difference of 0.19 to −0.17. Cronbach’s α values for the test—retest sessions were 0.945, 0.919, 0.872, and 0.791 at 0.25°, 0.50°, 1.00°, and 1.75° foveal eccentricity, respectively.

**DISCUSSION**

Table 2 compares the MPOD measurement in the present study with those in other studies published in the literature. We included only studies that in which HFP was used to measure the MPOD. After statistically comparing our data with results from the other studies (approximately one half U.S.-based
and the other half from other countries), we found our study to have significantly higher MPOD than that found in the U.S.-based studies\textsuperscript{22,28,30–41} (0.50 vs. 0.32, \(P = 0.0027\)), whereas, compared with studies from other countries,\textsuperscript{1,8,11,19,27,29,42–46} results were not significantly different (0.50 vs. 0.38, \(P = 0.056\)). This is the first report of MPOD in an Indian sample. The MPODs were higher in our Indian sample than in other studies. The difference can be attributed to higher intakes of a vegetarian diet rich in carotenoids (Hammond BR, et al. \textit{IOVS} 2002;43:ARVO E-Abstract 3604). Ciulla et al.\textsuperscript{28} have shown that individuals with brown-black irises have 19% higher MPOD than those with blue-gray irises. South Indian populations have predominantly brown-black irises, which may also explain the higher MPOD in the Indian population.

The macular pigment level increased up to the 30- to 39-year age group and then declined at all eccentricities. Similar to our findings, Lam et al.\textsuperscript{21} also found that the highest average MPODs were recorded in the 40- to 59-year age group. Thus, there is an increase in MPOD up to 40 years. Thereafter, we found a decline in MPOD in all age groups and in both sexes. This negative correlation of age and MPOD has been seen in other studies.\textsuperscript{11,21,33} The age-related decline in MPOD could be due to the excessive depletion because of utilization of lutein.

### Table 1. Relationship of Mean MPOD in the Various Age Groups in Either Sex

<table>
<thead>
<tr>
<th>Foveal Eccentricities</th>
<th>Mean ± SD (Log Units)</th>
<th>Mean ± SD (Log Units)</th>
<th>Mean ± SD (Log Units)</th>
<th>ANOVA</th>
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<tr>
<td></td>
<td>(0.25^\circ)</td>
<td>(0.50^\circ)</td>
<td>(1.00^\circ)</td>
<td>(1.75^\circ)</td>
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<td>Overall</td>
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<tr>
<td>Male</td>
<td>0.56 ± 0.24</td>
<td>0.428</td>
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<td>Female</td>
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<td>0.422</td>
<td>0.49 ± 0.20</td>
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<td>20–29</td>
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<tr>
<td>Male</td>
<td>0.66 ± 0.13</td>
<td>0.159</td>
<td>0.54 ± 0.14</td>
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<tr>
<td>Female</td>
<td>0.60 ± 0.18</td>
<td>0.180</td>
<td>0.47 ± 0.18</td>
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<tr>
<td>30–39</td>
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<tr>
<td>Male</td>
<td>0.74 ± 0.25</td>
<td>0.704</td>
<td>0.56 ± 0.26</td>
<td>0.716</td>
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<tr>
<td>Female</td>
<td>0.72 ± 0.19</td>
<td>0.724</td>
<td>0.54 ± 0.20</td>
<td>0.578</td>
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<tr>
<td>40–49</td>
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<tr>
<td>Male</td>
<td>0.75 ± 0.28</td>
<td>0.039</td>
<td>0.56 ± 0.25</td>
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<tr>
<td>Female</td>
<td>0.62 ± 0.18</td>
<td>0.180</td>
<td>0.48 ± 0.20</td>
<td>0.182</td>
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<tr>
<td>50–59</td>
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<tr>
<td>Male</td>
<td>0.57 ± 0.21</td>
<td>0.019</td>
<td>0.45 ± 0.19</td>
<td>0.041</td>
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<tr>
<td>Female</td>
<td>0.71 ± 0.22</td>
<td>0.041</td>
<td>0.55 ± 0.18</td>
<td>0.167</td>
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<td>≥60</td>
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<tr>
<td>Male</td>
<td>0.52 ± 0.24</td>
<td>0.658</td>
<td>0.45 ± 0.25</td>
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<tr>
<td>Female</td>
<td>0.49 ± 0.23</td>
<td>0.594</td>
<td>0.39 ± 0.21</td>
<td>0.266</td>
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Post hoc power calculation

Entries in bold indicate significant values (\(P \leq 0.05\)).
and zeaxanthin due to the age-related increase in oxidative stress.43,45 It could also be due to inadequate accumulation of macular carotenoids, because of age-related changes in dietary intake, absorption, and transport of serum carotenoid.46,47 However, there are studies that show no effect of age on MPOD.20

**Figure 4.** Bland-Altman plots showing differences between eccentricities by average of repeat and first session of MPOD measurements.
Recently published studies pertaining to sex differences in MPOD are conflicting. Some studies report that males have higher MPOD than do females, whereas others deny any relationship. We found that the men in our study had higher MPOD than the women in the 40- to 49-year age group. Hammond et al. also reported higher MPODs in men. However, in the 50- to 59-year age group, the women had higher MPODs than did the men. These differences can be explained by hormonally controlled variations in the lipid transport system, which is used by carotenoids. Steroid hormones, may also affect the metabolism of carotenoids directly. Also women are known to have a higher percentage body fat, which is also known to affect MPOD.

We found an exponential decrease in MPOD with increasing eccentricity from the fovea. Our MPOD data showed a unimodal distribution that was similar to that in HFP studies by Snodderly et al., Berendschot and van Norren, Delori et al., Snodderly et al., Wooten and Hammond, Hammond et al., Lam et al., and Beatty et al. Whereas, studies performed by Berendschot and van Norren, Delori et al., and Wolf et al. showed a bimodal distribution with a central peak surrounded by a ring with high-density values of MPO. When measured with fundus reflectance, autofluorescence, and modified confocal scanning laser ophthalmoscopy.

We found no significant interocular difference in MPOD. This finding was consistent with those in previous reports. The subjects did not show any significant difference in sex or age. Our study is unique, as it is the first report of MPOD distribution in a sample of the Indian population. This is important, as there is evidence that age-related macular degeneration is less prevalent in the Indian subcontinent. Unlike other studies, the distribution in various age groups and between the sexes was uniform in this sample.

The study had some limitations. Being a descriptive study, we did not examine the factors that might influence MPOD levels except age and sex. The increased level of melanin in darker eyes, especially near the fovea might alter the measurement of MPOD and provide a lower MPOD. Possible artifacts that might have influenced the data include subjects who did not turn the knob themselves. In conclusion, this study establishes the MPOD normogram in an Indian sample. This information will be useful in understanding the epidemiology and presentations of age-related macular degeneration in this population.

References
