Effect of Cataract in Evaluation of Macular Pigment Optical Density by Autofluorescence Spectrometry

Yuzuru Sasamoto, Fumi Gomi, Miki Sawa, Hirokazu Sakaguchi, Motokazu Tsujikawa and Kohji Nishida

PURPOSE. To assess the effect of cataract on the evaluation of macular pigment optical density (MPOD) in aged patients.

METHODS. MPOD was prospectively measured using autofluorescence spectrometry before and after cataract surgery. The Lens Opacities Classification System III was used to grade the cataracts at baseline.

RESULTS. Forty-five eyes of 41 subjects, who had no ocular disorders or fundus autofluorescence abnormalities except for age-related nuclear cataract, were included. Preoperative MPOD was 0.350 ± 0.117 density unit (DU). Regression analysis showed that a higher nuclear color score correlated with lower MPOD (t = −2.90, P = 0.0065). The preoperative MPOD prediction formula was MPOD = 0.545 − 0.069 × nuclear color score. A higher nuclear color score correlated significantly with failure to measure the MPOD (γ² = 5.08, P = 0.0242). The mean postoperative MPOD was 0.600 DU (95% confidence interval [CI], 0.562–0.637), which was significantly (P < 0.0001) higher than the preoperative level of 0.350 DU (95% CI, 0.313–0.388). Regression analysis showed that higher preoperative MPOD correlated with higher postoperative MPOD (t = 2.91, P = 0.0061).

CONCLUSIONS. Cataract, especially its nuclear component, affects MPOD measured by autofluorescence spectrometry. Care should be taken when using this method in eyes with age-related macular maculopathy and age-related macular degeneration and in older patients who may develop these diseases.

Invest Ophthalmol Vis Sci. 2011;52:927–932 DOI:10.1167/iovs.10-5664

Macular pigment comprises three carotenoids (lutein, zeaxanthin, and mesozeaxanthin) and has light-absorbing properties in the 400- to 540-nm range, with maximum absorption at approximately 460 nm. In addition, the macular pigment itself has an antioxidative effect. Thus, macular pigment helps retard some destructive processes in the retina and the retinal pigment epithelium, which may lead to macular diseases such as age-related maculopathy (ARM) and age-related macular degeneration (AMD). Some investigators have tried to measure the macular pigment to determine its relationship with the development of ARM or AMD. Several clinical methods of measuring macular pigment optical density (MPOD) have been used, including heterochromatic flickering photometry (HFP), motion-detection photometry, fundus reflectance spectroscopy, Raman spectrometry, and autofluorescence spectrometry. To date, factors such as sex, aging, race, smoking, and cataract are postulated to affect the density of the macular pigment; however, the data are not consistent among the published reports.

Cataract absorbs blue light, which damages the retina and may cause development of AMD. In addition to visual deterioration, the blue light-absorbing property of cataracts may affect the accuracy of the MPOD measurements. Autofluorescence spectrometry, which uses a two-wavelength method to measure MPOD, acquires fundus autofluorescence (FAF) images obtained at two wavelengths, 488 and 514 nm and then the subtraction of the logarithm of these images creates an MPOD map.

Since 488-nm is within the range of blue light, cataracts may reduce the signals in a 488-nm autofluorescence image. Thus, when we measure the MPOD in the eyes of elderly people, we should know how cataracts affect the results.

In the present study, we used autofluorescence spectrometry to measure the MPOD in eyes by using autofluorescence spectrometry before and after surgery to determine the effects of cataract.

METHODS

Study Population

We conducted a prospective interventional study at Osaka University Hospital from November 2008 to December 2009. The institutional review board approved the study.

Consecutive patients were enrolled who had no ocular disorders, including FAF abnormalities, except for age-related nuclear cataract. Subjects taking supplements containing lutein, zeaxanthin, and/or beta carotene were excluded. The patients underwent standard phacoemulsification and implantation of a yellow-tinted intraocular lens (IOL) (AcrySof SN60WF; Alcon Laboratories, Fort Worth, TX). No complications or adverse events occurred during cataract surgery or the follow-up period. In accordance with the Declaration of Helsinki, all participants provided informed consent before MPOD was measured.

Measurement and Analysis of MPOD

We used modified angiography (Heidelberg Retina Angiograph [HRA], Heidelberg Engineering, Dossenheim, Germany) to measure MPOD in all eyes before and after cataract surgery. The principle of measurement of MPOD with autofluorescence spectrometry with the two-wavelength method has been published. All measurements were performed by two masked orthoptists, who used the same testing device and protocol. Before the study, the reliability of the measurements between two orthoptists was confirmed as reported previously. MPOD was measured within 1 week before cataract surgery and within 2 weeks after cataract surgery.
Before the measurement, sufficient pupil dilation was obtained by instillation of dilating drops containing 0.5% tropicamide and 2.5% phenylephrine. Subjects sat before a table and fixated with the fellow eye on an external light source. If the fellow eye did not have adequate visual acuity (VA) for fixation, the subjects were asked to look straight as much as possible. The modified angiograph was aligned with the subject’s eye, and movies were taken with the 488- and 514-nm excitation wavelengths (scan size, 30°); computed mean autofluorescence images were obtained at each wavelength, and the two images were subtracted to calculate the MPOD (expressed as the density unit [DU]). The mean MPOD, averaged along the area of an annulus with a retinal eccentricity of 0.5° (1° circle at the fovea), was selected as the value representing the MPOD. We measured the MPOD two or three times in each eye during each visit and then selected the data with the best-qualified image.\(^{25}\)

The signal intensities of each 488- and 514-nm autofluorescence image were displayed in gray scale, with levels from 0 to 255. Delori et al.\(^{25}\) reported the following equation for calculating MPOD, in which \(K\) is a constant, and \(F_p\) and \(F_f\) are the gray level in the perifoveal area (6° circle) and the fovea (0.5° circle), respectively.

\[
\text{MPOD} = K \left[ \frac{\log[F_p(488 \text{ nm})/F_f(488 \text{ nm})]}{F_p(514 \text{ nm})/F_f(514 \text{ nm})} - \log[F_p(514 \text{ nm})/F_f(514 \text{ nm})] \right]
\]

To record the components while calculating the MPOD, we manually measured the \(F_p\) and \(F_f\) in the 488- and 514-nm images before and after surgery in all patients and converted the values into a logarithmic scale referring to the above formula and described the results as the mean (95% CI). The distribution profiles of gray levels of 20 × 20 pixels at the peripheral retina were also obtained, and the skew of the distribution was estimated by calculating the following equation

\[
\text{Skew} = \frac{(GL_{5\%} + GL_{95\%})/2 - GL(\text{mode})}{GL(\text{mode})}
\]

where \(GL_{5\%}\) and \(GL_{95\%}\) are the 5th and 95th percentiles of the gray level (GL), and GL(mode) is the most frequently occurring gray level. The skew, which is considered to be one of the indexes of random noise of the autofluorescence image, was also described as the mean (95% CI).

**Ophthalmic Examination**

The clinical examinations included best corrected VA (BCVA) determined with Landolt C charts, intraocular pressure, slit lamp biomicroscopy, and ophthalmoscopy with lens and fundus photographs taken before and after cataract surgery. The slit lamp digital images were used to assess the type and the severity of the lens opacities. Two masked ophthalmologists (YS and FG) graded the nuclear opalescence, nuclear color, and cortical and posterior subcapsular cataracts in each eye based on the Lens Opacities Classification System III (LOCS III).\(^{34}\)

The nuclear opalescence and color in the observed lens were classified into standards 1 through 6 and scored from 0.1 to 6.9 (ranging from clear or colorless to very opaque or brunescent) by each observer and the average scores were chosen. Eyes with a cortical cataract were graded based on the area of the opacity. We defined eyes with a grade of more than 3.0 (area with opacity within the pupillary area exceeding 20%) as having a cortical cataract. Similarly, eyes with a posterior subcapsular cataract were graded on the basis of the size of the opacity. We defined eyes with a grade of more than 1.0 (obvious opacity at the center of the posterior lens) as having a posterior subcapsular cataract.

**Statistical Analysis**

Since variations in an individual patient were nearly equivalent to those between patients, all eyes in which the MPOD was measured were included in the analysis, even if both eyes of a patient underwent cataract surgeries. The BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) for the statistical analysis. In comparing the mean logMAR VA and MPOD before and after cataract surgery, the 95% CI was calculated, and a paired \(t\)-test was performed.

To estimate the difference in MPOD between subgroups, we analyzed the following parameters by \(t\)-test: sex, smoking history, and presence of a cortical cataract and posterior subcapsular cataract. The correlation between the MPOD and age, nuclear opalescence score, nuclear color score, and the logMAR VA, and the correlation between nuclear opalescence or color score and logMAR VA was assessed by calculating Pearson’s correlation coefficient (\(r\)). Stepwise regression analysis using the Akaike information criterion was performed to determine the variables that affected MPOD before cataract surgery and the difference between MPOD before and after cataract surgery: sex, age, smoking, nuclear opalescence and color score, cortical cataract, posterior subcapsular cataract, and preoperative logMAR VA. When stepwise regression was performed to determine the variables that affected MPOD after cataract surgery, we included sex, age, smoking, postoperative logMAR VA, and preoperative MPOD as explanatory variables. Stepwise regression analysis was also conducted, to determine the variables that correlate with failure to obtain reliable MPOD data.

To estimate the difference in gray levels of the perifoveal area (\(F_p\)) and the fovea (\(F_f\)) before and after cataract surgery, we performed a paired \(t\)-test. The difference in \(F_p/F_f\) before and after surgery at 488 and 514 nm was also estimated by paired \(t\)-test. Pearson’s correlation coefficient (\(r\)) was used to determine the correlation between the nuclear color score and the skew index. \(P < 0.05\) was considered significant (JMP software version 8.0; SAS Institute Inc., Cary, NC).

**RESULTS**

**Baseline Characteristics and MPOD**

A total of 45 eyes of 41 subjects (16 men, 18 eyes; 25 women, 27 eyes) were included. The baseline characteristics of all eyes are shown in Table 1. The mean age ± SD was 71.6 ± 6.7 years. Sixteen patients (18 eyes) were smokers, and 25 (27 eyes) were nonsmokers. The mean ± SD nuclear opalescence and color scores were 2.9 ± 0.9 and 3.0 ± 0.9, respectively, and they correlated very strongly with each other (\(r = 0.97, P < 0.0001\)). The logMAR BCVA (mean ± SD) was 0.39 ± 0.29. The mean preoperative MPOD measured by autofluorescence spectrometry was 0.350 ± 0.117 DU. We could not obtain reliable data (200/225 pixels) in five eyes and excluded them from the preoperative evaluation.

MPOD before cataract surgery is summarized in Table 2. It was significantly reduced, in parallel with the increases in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects/eyes, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women, n subjects</td>
<td>16/25</td>
</tr>
<tr>
<td>Men/women, n eyes</td>
<td>18/27</td>
</tr>
<tr>
<td>Age, mean ± SD</td>
<td>71.6 ± 6.7</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>16/25</td>
</tr>
<tr>
<td>Yes/no, n subjects</td>
<td>18/27</td>
</tr>
<tr>
<td>Yes/no, n eyes</td>
<td>16/25</td>
</tr>
<tr>
<td>Nuclear scores by LOCS III, mean ± SD</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Opalescence</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td>Color</td>
<td>20/25</td>
</tr>
<tr>
<td>Cortical cataract, yes/no, n eyes</td>
<td>13/52</td>
</tr>
<tr>
<td>Preoperative MPOD measured by autofluorescence spectrometry, DU</td>
<td>0.350 ± 0.117</td>
</tr>
<tr>
<td>Subjects with unreliable data, n</td>
<td>5</td>
</tr>
</tbody>
</table>
MPOD decreased 0.069 DU. If the nuclear color score increased 1 point, factors that were correlated with failure to measure MPOD. A higher nuclear color score correlated significantly with failure to measure the MPOD ($\chi^2 = 5.08, P = 0.0242$). A posterior subcapsular cataract also tended to correlate with failure to measure the MPOD ($\chi^2 = 3.25, P = 0.0713$).

### Postoperative MPOD

The mean postoperative logMAR VA was $-0.002$ (95% CI, $-0.058$ to $0.054$), and it improved significantly compared with the preoperative logMAR VA of $0.394$ (95% CI, $0.306$–$0.481$; $P < 0.0001$).

We could obtain MPOD data in all eyes after cataract surgery. A representative case was shown in Figure 1. The mean postoperative MPOD was $0.600 \pm 0.124$ DU (95% CI, $0.562$–$0.637$). The postoperative MPOD was significantly higher than the preoperative level of $0.350 \pm 0.117$ DU (95% CI, $0.313$–$0.388$; $P < 0.0001$). Postoperative MPOD correlated positively with the preoperative level ($r = 0.43, P = 0.0058$; Fig. 2).

MPOD data after cataract surgery are summarized in Table 3. The MPOD tended to be higher in the women (0.626 [95% CI, 0.582–0.670]) than in the men (0.559 [95% CI, 0.513–0.600]).

### Table 2. The MPOD Level before Cataract Surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MPOD (95% CI) (DU)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>0.316 (0.241–0.391)</td>
<td>(n = 16) 0.373 (0.333–0.413)</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>0.322 (0.261–0.383)</td>
<td>(n = 15) 0.367 (0.320–0.414)</td>
</tr>
<tr>
<td>Cortical cataract, yes/no</td>
<td>0.356 (0.296–0.416)</td>
<td>(n = 18) 0.345 (0.294–0.397)</td>
</tr>
<tr>
<td>Posterior subcapsular cataract, yes/no</td>
<td>0.326 (0.219–0.432)</td>
<td>(n = 9) 0.357 (0.317–0.398)</td>
</tr>
</tbody>
</table>

### Coefficient Correlation ($r$) $P$

| Age (n = 40)                  | 0.05 | 0.7570   |
| Nuclear opalescence score by LOCS III (n = 40) | $-0.37$ | 0.0177   |
| Nuclear color score by LOCS III (n = 40) | $-0.39$ | 0.0142   |
| Preoperative logMAR VA (n = 40) | $-0.42$ | 0.0077   |

### Figure 1. Pre- and postoperative images from an eye of a 71-year-old woman.

(A) Preoperative color of the cataract nucleus was scored as 4.2 and the nuclear opalescence score as 4.0. (B) Fundus autofluorescence images captured at the 488-nm (left) and 514-nm (right) wavelengths before surgery. The sensitivity of the detector was set relatively high, and random noise was seen in both images. The macular pigment blocked more signals from the macula in the 488-nm image. (C) The subtraction of the logarithm of these images creates an MPOD map. Preoperative MPOD was 0.29 DU. (D) A yellow-tinted IOL implanted during the surgery. (E) Postoperative fundus autofluorescence images captured at 488-nm (left) and 514-nm (right) wavelengths. The proper setting of the sensitivity of the detector led to less random noise. (F) Postoperative MPOD improved to 0.66 DU.
CI, 0.493–0.626; P = 0.0758). In addition, it tended to be in proportion to age (r = 0.29, P = 0.0547). The postoperative level correlated positively with the preoperative one (r = 0.43, P = 0.0058). We conducted a stepwise regression analysis in which the model included sex, age, smoking, postoperative logMAR acuity, and preoperative MPOD as explanatory variables and postoperative MPOD as a response. The analysis showed that higher preoperative MPOD correlated with higher postoperative MPOD (r = 2.91, P = 0.0061).

Difference in MPOD before and after Surgery

Simple regression analysis revealed that eyes with lower preoperative MPOD and a higher nuclear color score correlated with a larger difference in MPOD before and after cataract surgery (r = −0.54, P = 0.0001 and r = 0.36, P = 0.0157, respectively). After conducting a stepwise regression analysis, we found lower preoperative MPOD and no presence of posterior subcapsular cataract correlated with a larger difference in MPOD (t = −4.51, P < 0.0001; t = −2.18, P = 0.0355, respectively).

Gray Levels and Skew before and after Surgery

Gray level at the perifoveal region (Fp) and the fovea (Fx) and their ratio (Fp/Fx) before and after cataract surgery presented in logarithmic scale are summarized in Table 4. Fp before and after surgery did not change significantly at both 488 and 514 nm (P = 0.4167, P = 0.6848, respectively). Fx at 514 nm also did not show a significant change (P = 0.3808), but Fx at 488 nm significantly decreased after surgery (P = 0.0136). In addition, the increase in Fp/Fx after surgery at 488 nm was significantly higher than that at 514 nm: 0.19 (95% CI, 0.16–0.22) and 0.07 (95% CI, 0.05–0.09), respectively (P < 0.0001).

The skew at the peripheral retina is also shown in Table 4. It was significantly higher before surgery than after in both the 488- and the 514-nm images (P = 0.0001 and P < 0.0001, respectively). Statistical analysis showed that the higher skew index at 488 nm correlated moderately with higher nuclear color score (r = 0.45, P = 0.0028), but skew at 514 nm did not correlate with the nuclear color score (r = −0.02, P = 0.8995).

Table 3. The MPOD Level after Cataract Surgery

<table>
<thead>
<tr>
<th>MPOD (95% CD) (DU)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>0.559 (0.493–0.626) (n = 18)</td>
</tr>
<tr>
<td>Smoking, yes/no</td>
<td>0.573 (0.507–0.658) (n = 18)</td>
</tr>
</tbody>
</table>

Coefficient Correlation (r) | P    |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (n = 45)</td>
<td>0.29</td>
</tr>
<tr>
<td>Postoperative logMAR VA (n = 45)</td>
<td>0.18</td>
</tr>
<tr>
<td>Preoperative MPOD (n = 40)</td>
<td>0.43</td>
</tr>
</tbody>
</table>

DISCUSSION

We used autofluorescence spectrometry to measure MPOD in eyes with age-related cataract, before and after cataract surgery, and observed the significant increase in MPOD after cataract surgery. Mean MPOD was 0.350 DU (95% CI, 0.313–0.388) before surgery and 0.600 DU (95% CI, 0.562–0.637) after surgery. Considering the result from the previous report that the intensity of macular pigment is stable without intervention, the increased MPOD observed after cataract surgery must be artifactual. Therefore, we examined the factors that correlated with MPOD.

MPOD significantly increased after the cataractous lenses were removed and replaced with yellow IOLs, the color of which was similar to the lens color in subjects in the third decade of life. Preoperative MPOD tended to be lower when the nuclear opalescence and color scores were high, although there was no correlation between preoperative MPOD and cortical cataracts or posterior subcapsular cataracts. As expected, the nuclear opalescence score and the nuclear color score correlated very strongly (r = 0.97, P < 0.0001), but stepwise regression analysis showed that the nuclear color score was the most significant factor that affected preoperative MPOD (t = −2.90, P = 0.0063). In addition, we found in a simple regression analysis that lower preoperative MPOD and a higher nuclear color score correlated with a larger difference in MPOD. From these results, we concluded that the lower MPOD in the eyes with cataract was, to some degree, due to the yellower lenses.

As a reason for MPOD reduction by the presence of cataract, first, we hypothesized that the lens yellowing might reduce the autofluorescence level in 488-nm (blue) wavelength directly because yellow absorbs blue. However, as the lens yellowing reduced the autofluorescence at the perifoveal area and the fovea equally, it seemed not to affect the final MPOD in subjects in the third decade of life. Preoperative MPOD tended to be lower when the nuclear opalescence and color scores were high, although there was no correlation between preoperative MPOD and cortical cataracts or posterior subcapsular cataracts. As expected, the nuclear opalescence score and the nuclear color score correlated very strongly (r = 0.97, P < 0.0001), but stepwise regression analysis showed that the nuclear color score was the most significant factor that affected preoperative MPOD (t = −2.90, P = 0.0063). In addition, we found in a simple regression analysis that lower preoperative MPOD and a higher nuclear color score correlated with a larger difference in MPOD. From these results, we concluded that the lower MPOD in the eyes with cataract was, to some degree, due to the yellower lenses.

As a reason for MPOD reduction by the presence of cataract, first, we hypothesized that the lens yellowing might reduce the autofluorescence level in 488-nm (blue) wavelength directly because yellow absorbs blue. However, as the lens yellowing reduced the autofluorescence at the perifoveal area and the fovea equally, it seemed not to affect the final MPOD in the equation used to calculate it. Then, we examined gray levels in all images and also the skew condition. Finally, we reached the following conclusions: Excitation and emission signals are scattered by cataractous lenses, and excitation signals are partially absorbed by yellow lenses. Hence, to obtain an image bright enough to evaluate MPOD, we had to make the...
sensitivity of the detector high in the cataractous eyes, and this adjustment resulted in higher reflectivity, as seen in Figure 1. As a result, $F_p$ before surgery was at almost the same level after surgery. However, the skew index, which substitutes for random noise, was higher before surgery than after surgery, because high sensitivity induced high levels. This result made the $F_p$ falsely high compared with the $F_i$ before surgery, especially at 488 nm, because foveal signals in 488 nm are small due to the macular pigment and are easily affected by the random noise. Consequently, the increase in $F_p/F_i$ after surgery at 488 nm was significantly higher than that at 514 nm and led to the decrease in MPOD before surgery. The statistical results, which showed a positive correlation between the skew and nuclear color score in the 488-nm wavelength images but not in the 514-nm wavelength images, may indicate that lens yellowing increases the random noise of the autofluorescence image, especially at 488 nm and, as a result, decrease the MPOD. Of course, we might consider other effects such as autofluorescence of the lens. However, as mentioned by Delori et al.,

```markdown
<table>
<thead>
<tr>
<th>Table 4. Grey Levels before and after Cataract Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before surgery</td>
</tr>
<tr>
<td>488-nm                                     1.33 (1.24–1.42)</td>
</tr>
<tr>
<td>514-nm                                     1.45 (1.34–1.52)</td>
</tr>
<tr>
<td>After surgery</td>
</tr>
<tr>
<td>488-nm                                     1.39 (1.29–1.50)</td>
</tr>
<tr>
<td>514-nm                                     1.48 (1.37–1.58)</td>
</tr>
</tbody>
</table>
```

Data are expressed as the gray level (95% CI).

In conclusion, our data suggest that cataracts affect the measurement of MPOD by autofluorescence spectrometry, probably as a result of the high setting of the detector’s sensitivity. Nuclear cataract seemed to associate with this high-sensitivity setting, producing a random noise especially in 488-nm wavelength images. Care should be taken when evaluating MPOD using this method in eyes with age-related macular maculopathy and macular degeneration and in patients who are old enough to develop these diseases. More quantitative and higher quality methods of averaging the reflectivity may yield a normalized autofluorescence image in which the effect of cataract is excluded and may allow more precise evaluation of MPOD.

**Acknowledgments**

The authors thank Thomas Fendrich (Heidelberg Engineering, Dossenheim, Germany) for pertinent and helpful advice.

**References**


