Fluorometry of the Crystalline Lens for Correcting Blue-on-Yellow Perimetry Results

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Purpose. Optical and neural sources of short wavelength sensitivity should be separated in the assessment of the results of blue-on-yellow (B/Y) perimetry. It has been shown previously that lens autofluorescence is related directly to lens yellowing and age. The aim of this study was to find out if B/Y perimetry results can be better corrected by using lens fluorometry than by age.

Methods. The authors evaluated one randomly chosen eye of 40 normal subjects and 39 age-matched patients with ocular hypertension and different stages of glaucoma. The authors obtained the mean sensitivity (MS) of the 24-2 B/Y visual fields with a Humphrey perimeter and determined the lens transmission index (LTI) from the ratio between posterior and anterior autofluorescence peaks measured with their fluorometer. A multiple regression analysis was used to evaluate the variability of the B/Y MS by age and LTI in normal subjects.

Results. The authors found a statistically highly significant linear correlation of B/Y MS to LTI in healthy subjects (R = 0.83; P < 0.0001). The 95% prediction interval of the normal subjects was determined. The majority of the MS values of the ocular hypertensives were inside the prediction limits, whereas approximately half of the patients (4 of 9) with early glaucoma and the majority of patients (14 of 15) with moderate and advanced glaucoma were below the prediction interval. The residual standard deviation of the B/Y MS with age alone was larger than that with LTI alone in the model (3.66 dB and 3.22 dB, respectively).

Conclusions. The interindividual variation of the lens transmission properties increases with age. The reference level for correcting B/Y perimetry results can be determined more precisely using fluorometry of the lens than with age alone. Invest Ophthalmol Vis Sci. 1997;38:697–703.

Recent studies have shown that the blue-on-yellow (B/Y) visual field testing shows deficits in glaucomatous eyes earlier and larger in size and depth than in standard white-on-white (W/W) perimetry.1–6 Although B/Y perimetry without correction of lens density shows greater specificity and sensitivity than does standard perimetry,7 short wavelength-sensitivity losses appear to have both a generalized and a localized component.4,8 Similarly, we know that in approximately half of eyes in which glaucoma is developing, the first detectable retinal nerve fiber layer abnormality is generalized thinning of retinal nerve fiber layer.9

To show the possible generalized component of damage, one must identify the extent of reduced blue transmission, which can be attributed to preretinal media. One can concentrate on lens transmission characteristics because the other preretinal medias show very little change in transmittance with age. For correcting lens transmission losses, recent studies have used a comparison of scotopic sensitivity with the known absorption curve of rhodopsin,10 but this procedure is time consuming. In addition, it is not clear how this method works in patients with damaged retina. A video-based method using a double-pass Purkinje image reflection technique11 and a technique using the retina as reflector for a double-pass measurement of lens density12 also have been reported.
Previous studies from our unit and from other investigators indicate that autofluorescence (AF) of the lens is related closely to aging and yellow coloration of the lens. The evaluation of the lens absorption by lens AF measurement was first proposed by Zeimer and Noth and later applied and refined by van Best et al. They assumed that the maximum AF is approximately the same in the anterior and posterior part because the lens is rather symmetrical in structure. Any difference in AF intensity between anterior and posterior parts then can be attributed to a loss of exciting and fluorescent light in the lens by scatter and by absorption in the nucleus and cortex.

In the conventional W/W perimetry, the threshold values are corrected for aging. In B/Y perimetry, however, the lens-induced absorption of the blue light may show large variability in equally old, healthy individuals. Therefore, the purpose of this study was to investigate whether there is a correlation between lens transmission indices obtained by fluorometry and B/Y visual field thresholds in individuals who are nonglaucomatous and whether fluorometry can be used in correcting B/Y perimetry results for lens yellowing.

METHODS

We evaluated one randomly chosen eye of 40 nonglaucomatous subjects with a mean age of 57 years (range, 29 to 84 years). Criteria for subject eligibility consist of normal findings in the ocular examination, a normal W/W visual field, no family history of glaucoma, no history of ocular or neurologic disease, no history of diabetes or other systemic diseases, and no history of any medications that are known to affect visual field sensitivity or color vision. No restriction was placed on contact lenses. The lowest visual acuity resulting from lens changes was 0.2.

In addition to nonglaucomatous subjects, we examined 39 patients (mean age, 59 years). Eleven patients had ocular hypertension (intraocular pressure of >22 mm Hg on three or more occasions) with normal optic disc, normal retinal nerve fiber layer, and normal W/W visual fields. Four ocular hypertensives had normal W/W visual fields but abnormal retinal nerve fiber layer or optic disc. Twenty-four patients had primary open-angle glaucoma and were divided according to W/W visual field defects: early glaucoma group in nine patients with a mean defect (MD) < 5 dB; moderate glaucoma in seven patients with MD between 5 and 10 dB; and advanced glaucoma in eight subjects with MD more than 10 dB.

The investigation followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all participants after the nature of the study had been explained.

The AF of the lens was measured using a fluorometer designed, built, and clinically tested in our department. The schematic representation of the optical system of our fluorometer and technique of lens fluorometry has been described previously. The excitation light source is a 25-W incandescent lamp from Zeiss biomicroscope fed by a current-regulated power supply. Infrared light is blocked by an absorption filter. The wavelength of excitation light is set by an interference filter with peak transmission at 495 nm. A barrier interference filter has peak transmission at 520 nm. Transmission characteristics of these filters are shown in the previous article.

The optical system consists of a moving motorized lens and a fixed lens that focus the excitation light on the eye and collect emitted light from the eye. The dimensions of the illumination slit measured at the focal plane in air are 0.1 × 1.0 mm. The beam is scanned linearly along the optical axis of the eye at an angle of 20° while the subject views a fixation target. Fluorescence emitted from the lens passes the lens system on the opposite side at a symmetrical angle and is transmitted through a slit and a barrier filter to a photodiode detector (EG&G Electro-Optics, Salem, MA: UV-040 BG, spectral range, 250 to 1150 nm). Any blue light due to scatter within the eye is reflected from the barrier filter and focused in front of the ocular.

The AF measured as a function of the distance in the eye is processed by low-noise amplifiers, digitized, stored, and plotted on the computer screen. The device produces a graphic fluorescence profile that consists of anteroposterior juxta cortical peaks and a central plateau (Fig. 1). A transmission index was calculated from the ratio between the heights of the posterior and anterior AF peaks. The coefficient of variation for the lens transmission index is 2.9%.

Each unidirectional scan from the vitreous to the anterior chamber takes approximately 3 seconds. No contact lens is used. The instrument is calibrated before each measurement using a fluorescent reference surface. The long-term stability curves of the entire fluorometer show good stability at two different fluorescence levels as shown in the previous article.

We obtained both W/W and B/Y visual fields on a modified Humphrey Field Analyzer (model 610; Humphrey Instruments, San Leandro, CA) using program 30-2. Details of the B/Y test procedure have been provided in several previous publications. B/Y perimetry was performed with a 100 cd/m² yellow background and a size V blue (440 nm) stimulus. In nonglaucomatous subjects, the W/W perimetry was carried out during the first visit.

To avoid the possible depression in midperiphery associated with lack of perimetric experience, we decided apart from total mean sensitivities (MSs) to in-
Lens Autofluorescence and Blue-on-Yellow Perimetry

Aiscenceative fluorescence

FIGURE 1. A typical autofluorescence profile showing the anterior (A) and posterior (P) autofluorescence peaks. Lens transmission index was calculated from the ratio of the posterior peak to the anterior peak.

include in the analysis the additional 12 points, three in each quadrant, four points at approximately 12.7° from fixation, one of the coordinates 9°/−9° and the other 9°/−9°, respectively, and eight points at approximately 9.5° from fixation, one of the coordinates 9°/−9° and the other 3°/−3°, respectively (9° eccentricity pattern). The four most central points were excluded because of the possible interindividual variation in macular pigmentation. For achieving data for program 24-2 test, we subtracted respective peripheral location values from program 30-2 data.

The statistical analysis of the data was performed using a SOLO software package (BMDP Statistical Software, Los Angeles, CA). The relation among lens transmission index, B/Y visual field threshold values, and age was analyzed using regression technique. To evaluate the variability of the B/Y mean sensitivity by age and lens transmission index in healthy subjects, we performed a multiple regression analysis with MS as the dependent variable and age and lens transmission index as the independent variables. The software SPSS 6.1.2. for Windows (SPSS, Chicago, IL) was used in regression computations.

RESULTS

The total MS values of the visual field programs 30-2 and 24-2 as well as the MS of 9° eccentricity pattern showed a statistically highly significant correlation with the lens transmission index in nonglaucomatous individuals (R = 0.81, 0.83, and 0.86, respectively; P < 0.0001). Because the differences between the correlation coefficients clinically were not significant, we decided to present here the program 24-2 data because of the program's practical applicability and general acceptance for visual field testing.

The correlation of B/Y visual field (program 24-2) MS values in nonglaucomatous individuals plotted against the lens transmission index is shown in Figure 2. It is a linear relation with the data best fitted by the equation:

\[ Y = 8.56 + 0.173 \times X \]

where X is the lens transmission index and Y is the expected total MS in healthy individuals. We also determined the 95% prediction interval for the individual measurements of B/Y MS values as a function of the lens transmission index in nonglaucomatous subjects.

FIGURE 2. The correlation of the blue-on-yellow visual field mean sensitivity values (program 24-2) in nonglaucomatous individuals plotted against the lens transmission index. There is a statistically highly significant correlation (R = 0.85; P < 0.0001). The 95% prediction interval is shown.
FIGURE 3. The relation of the blue-on-yellow visual field mean sensitivity values to the lens transmission index in the ocular hypertensive groups with the 95% prediction interval of the normal subjects. The circles represent patients with ocular hypertension who had an intraocular pressure >22 mm Hg with normal optic disc, normal retinal nerve fiber layer, and normal Humphrey 30–2 white-on-white visual fields. The triangles represent patients with ocular hypertension with normal Humphrey 30–2 visual fields but abnormal retinal nerve fiber layer or optic disc.

expected and measured MS values in patients with glaucoma, that is, the age-corrected MD in B/Y perimetry in patients with glaucoma (Fig. 6).

We also calculated the difference between expected and measured MS values for patients with glaucoma using the regression equation of B/Y mean sensitivity to lens transmission index in normal subjects, that is, the MD in B/Y perimetry corrected for lens transmission index in patients with glaucoma (Fig. 6).

The distribution of the obtained MD values calculated by age and by lens transmission index in patients with glaucoma is shown in Figure 6. Scatterplot of the lens transmission index on age is shown in Figure 7. There was a statistically highly significant correlation (R = 0.80; P < 0.0001).

The regression analysis with MS value as the dependent variable indicated that lens transmission index provided a more precise prediction of the MS value than did age. The residual standard deviation of the regression model including age alone was 0.44 dB larger than that having lens transmission index as the independent variable (standard deviation, 3.66 and 3.22 dB, respectively). Adding the lens transmission index to the model including only age reduced the residual standard deviation by 0.57 to 3.09 dB (P = 0.003). However, the residual standard deviation was reduced only by 0.13 dB (P = 0.04) when age was added to the model containing lens transmission index only.

DISCUSSION

Recently published prospective studies have reported that visual field defects are identified earlier using B/Y perimetry than by W/W perimetry.1–6 However, the major disadvantage of B/Y perimetry is that transmission properties of the lens should be measured to separate optical and neural sources of short wavelength sensitivity loss. In this study, we have presented a procedure that provides a practical estimate of absorption of the blue light in an individual lens.

The scatterplot of the patients with ocular hypertension (Fig. 3) shows that in 11 of 15 patients, the MS values fall inside the limits of the 95% prediction interval, suggesting that their B/Y mean sensitivity values are not different from those of the normal subjects. Without the knowledge of the lens transmission properties, many of the lower MS values erroneously could have been interpreted as abnormal. Given that patients with ocular hypertension have normal B/Y visual fields, they would have only a 5% chance of being outside the limits of the 95% prediction interval.

The MS values in the early glaucoma group were divided between the ones inside (five patients) and the ones outside (four patients) of the prediction interval of the normal subjects. This suggests that on the basis of the MS values of their B/Y visual fields, the five patients within the prediction interval are not different from our normal subjects or most of our patients with ocular hypertension. However, the four patients with MS values below the lower reference level would be glaucomatous measured both with W/W perimetry and with B/Y perimetry with lens AF taken into account.

In the moderate and advanced glaucoma groups,
FIGURE 5. The blue-on-yellow mean sensitivity values in normal control subjects (program 24-2) showed a good correlation to age ($R = -0.77; P < 0.0001$).

FIGURE 6. Scattergram showing the relation between the blue-on-yellow mean defects in patients with glaucoma corrected for their age and lens transmission index. The deviations from the linear line show differences between two ways of correction.
14 of 15 patients had an MS value below the lower reference limit (Fig. 4), suggesting that they indeed have a true optic nerve damage and their low B/Y perimetry thresholds are not determined by lens yellowing alone.

The regression of B/Y MS value (program 24-2) on lens transmission index in the normal subjects (Y = 8.56 + 0.173 X X) can be used to estimate the lens transmission index-corrected MD of B/Y perimetry in patients with ocular hypertension and glaucoma.

Previous reports have shown that there is a good correlation between lens transmission values and age. From this point, the question arose whether performing the lens transmission property measurement adds information beyond that provided by age alone. To examine this, we fitted the regression model to the perimetry data with both age and lens transmission index together and separately. The residual standard deviation of the B/Y mean sensitivity with the lens transmission index alone was smaller than with age alone. Adding the lens transmission index to the regression model reduced the residual standard deviation considerably more than adding age to the model. Therefore, the B/Y mean sensitivity data are less variable with the lens transmission index, and we can conclude that the lens-corrected MD is more precise than is the age-corrected MD.

A good correlation between lens transmission values and age also was found in this study. However, the interindividual variation of the lens transmission properties increases considerably with age (Fig. 7). Therefore, the average values will not serve well for individual cases. To illustrate this statement, we calculated the MD for patients with glaucoma using two different equations. If the two techniques were identical, the MD values in Figure 6 would be on or close to the diagonal. Figure 6 shows, however, that if thresholds in patients in whom the individual lens transmission properties were better than expected according to age, the age-corrected mean defects tend to be underestimated. And reverse, if the patients' lens transmission properties are worse than expected, the age-corrected MD values tend to be overestimated.

One might postulate that before being able to decide whether a retinal sensitivity to blue stimulus is abnormal, not only the lens transmission properties but also the physiological behavior of retina with age must be taken into account. According to previous studies, the age-related decrease in sensitivity of short-wavelength-sensitive cone pathways is 0.05 to 0.15 log units per decade. However, regarding the extent of obtained prediction intervals (i.e., interindividual variation), the possible influence of neural aging to threshold estimation seems to be quite small. Actually, the correlation between the lens transmission index and the MS indirectly includes the aging factor because the older subjects have lower lens transmission values.

The results of this study suggest that the predic-
tion interval of B/Y mean sensitivity on lens transmission index may be used to estimate the effect of lens yellowing on the B/Y perimetry. The accuracy of the predicted interval will increase by examining a large number of normal subjects.

Key Words
blue-on-yellow perimetry, glaucoma, lens autofluorescence, lens yellowing, normal subjects

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References