Luminance-modulated adaptation in the global flash mfERG: A preliminary study of early retinal functional changes in high risk glaucoma patients

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Abstract

Purpose: To investigate the association of the luminance-modulation global flash multifocal electroretinogram (mfERG) and other clinical assessments of vision in subsets of subjects at high risk of developing glaucomatous damage.

Methods: Eighteen subjects (28 eyes) with asymmetric glaucoma and ocular hypertension were measured in this longitudinal study of visual field, OCT and multifocal electroretinogram (mfERG). Five ophthalmic examinations were scheduled, once every 12 months over a four-year period. The mfERG was assessed using a luminance-modulated global flash stimulation paradigm. The adaptive index which we have reported previously was calculated.

Results: There was a significant thinning of the peripapillary retinal nerve fiber layer over the course of the study for eyes with ocular hypertension, or for fellow eyes with asymmetric glaucoma which initially had an abnormal adaptive index; such eyes showed a thinning rate of -3.59 and -3.69 μm/year respectively. However, no significant thinning was found for eyes which initially had a normal adaptive index. Two subjects were shown to have glaucomatous damage, confirmed by abnormal thinning of the retinal nerve fiber layer and visual field loss respectively at the last visit. However, these patients had shown an abnormal adaptive index in the mfERG measurement at the first visit.

Conclusions: The adaptive index calculated from the measurement of luminance-modulated global flash mfERG is useful for predicting progression of signs related to glaucoma, especially in high risk groups. The abnormal adaptive index reflects the

change in fast-adaptive mechanisms in the retina and indicates the risk of developing glaucoma.

Introduction

Primary open-angle glaucoma (POAG) is a chronic eye disease with loss of retinal ganglion cells,[1] and patients with POAG are usually asymptomatic in the early stage of this slowly progressive condition. There are several risk factors for developing POAG and the prevalence of glaucoma is higher in some subsets of the population. Patients with unilateral glaucomatous visual field loss are believed to constitute a high risk group for developing similar damage in the fellow eye,[2-6] since POAG is generally a bilateral but asymmetric disease. Ocular hypertension is defined as elevated intraocular pressure (IOP) without the visual field abnormalities which characterize glaucoma. Although elevated IOP is not always an indicator of glaucoma, it is a major risk factor in glaucomatous optic neuropathy.[7, 8] The risk is approximately 1% in those with IOP below 20 mmHg, and may be six times higher in those with IOP greater than 24 mmHg.[9]

Automated perimetry is the commonly accepted clinical test of visual sensitivity and reduction of visual sensitivity results from loss of retinal ganglion cells in glaucoma.[10] However, the relationship between losses in visual sensitivity and losses in retinal ganglion cells has not been precisely defined. It has been reported that an initial 5 dB loss in visual field sensitivity occurs only after about 25% of retinal ganglion cells have been lost.[11] To optimize early diagnosis and evaluation of the

progression of glaucoma, a functional test that can detect glaucomatous damage earlier than the currently available assessment tools is needed, as the loss of visual function can be minimized through timely therapy.

Different paradigms of the multifocal electroretinogram (mfERG) have been used to assess the retinal function for some years,[12-17] and the global-flash mfERG was developed to enhance inner retinal response contributions by emphasizing retinal fast-adaptive mechanisms.[18] The global flash paradigm consists of a periodic global flash interleaved with the pseudorandom binary m-sequence multifocal stimulation. This flash sequence produces two components, a direct component (DC) and an induced component (IC); the DC is analogous to a standard mfERG response and reflect certain light adaptation produced by the periodic global flashes,[19] while the IC is the change in the response to the global flash produced by the prior local flash, and has been reported to be affected in glaucoma.[20-23] Naso-temporal response asymmetry across the retina is reported for the IC of the human global flash mfERG, which is a nonlinear response component predominantly from the inner retina.[18] The IC can reflect impaired adaptive effects due to damage from glaucoma,[21, 24] but the sensitivity of localized IC changes in assessing glaucomatous damage in individual patients is reported to be limited.[25]

To quantify retinal adaptation, our group derived an index from the DC induced by luminance-modulated global flash mfERG, it is calculated by subtracting the area under the line joining the responses from high (2.12 cd·s/m^2) to low (0.62 cd·s/m^2) luminance-difference from the area under the luminance-modulated response function fitted with a second-order best-fit line of the DC responses, which indicates the dependence on the luminance-difference in the luminance-modulated response function.[23] This adaptive index was demonstrated to be sensitive to glaucomatous damage and correlated well with the localised visual field defect in individual patients. We have assumed that reduction of the adaptive index was related to abnormal retinal adaptive mechanisms, presumably resulting from inner retinal damage.[23, 26] Moreover, the index is reduced in fellow eyes in subjects with asymmetric glaucoma even when both retinal function and structure are within the normal range.[22] Hence, the adaptive index appears likely to predict the impairment of visual function in glaucoma.

In this study, the co-variation of factors which are known to be associated with glaucoma was tested over a period of four years. It is known that Optical Coherence Tomography (OCT) measures thinning of the retinal nerve fiber layer in glaucoma patients,[27] and the adaptive index is associated with visual field losses; this study was therefore conducted to determine if these factors co-vary in populations known to be

at risk of developing glaucoma. This study would provide additional data on reliability of these tests over this time period, and to indicate their clinical utility.

Methods

Subjects

Twenty-eight subjects with asymmetric glaucoma and ocular hypertension were enrolled in this longitudinal study which began in 2004-2005. Ten asymmetric POAG patients with unilateral glaucomatous visual field defects were enrolled and only eight of these completed the study; these eight subjects were aged from 29 to 59 years (mean 48.1 ± 9.7 years), with corrected visual acuity (VA) 0.1 logMAR (20/25) or better. All of these subjects had abnormal glaucomatous visual field loss in the affected eye, and normal visual fields in the fellow eye; the fellow eyes with normal visual field were selected for testing in this study. All subjects had asymmetric glaucoma of more than one year duration as diagnosed by their ophthalmologists and were being treated with either Latanoprost (Pfizer) or Timolol Maleate (Alcon) in both eyes throughout the study.

Eighteen ocular hypertensive subjects with IOP ≥ 22 mmHg (measured by Goldmann tonometry) were enrolled; only 10 of these subjects completed the study; they were aged from 16 to 59 years (mean 43.1 ± 14.4 years), with corrected VA 0.0 logMAR (20/20) or better. Corneal thickness of these subjects was measured using the Orbscan II optical pachometry system (Orbtek, Inc., Salt Lake City, UT) to account for the effect of central corneal thickness on tonometry. All IOP values were

corrected using the formula: P = A + (550 – T) / X, where P = IOP in mmHg, A = Goldmann applanation reading in mmHg, T = central corneal thickness in μm, X = the correction ratio.[28] The mean IOP of all eyes was 23.3 ± 0.86 mmHg after compensation for the central corneal thickness.

All participants underwent an eye examination before the study to exclude ocular abnormalities other than the inclusion criteria. All subjects had open anterior angles. Visual field measurements were conducted on all subjects using the central 30-2 threshold (SITA) test of the Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Inc., Dublin, CA). No tested eye showed a glaucomatous visual field defect.

In addition, all tested eyes had peripapillary retinal nerve fiber layer thickness within the age-matched normal range limits as measured by OCT (Carl Zeiss Meditec, Inc., Dublin, CA). Peripapillary retinal nerve fiber layer thickness was measured using the fast peripapillary retinal nerve fiber layer circular scan provided by OCT. The results consisted of the average of three concentric scans of the optic disc in the one measurement session, at a diameter of 3.46 mm and for the full 360° around the optic nerve head. The average of the three scans was then calculated automatically, and only the results with signal value greater than 7 were used. Scans were further analysed for each eye using the retinal nerve fiber layer thickness average analysis

which quantifies the average nerve fiber layer thickness in 12 different clock-hour sectors.

All research procedures adhered to the tenets of the Declaration of Helsinki and were approved by the Ethics Committee of The Hong Kong Polytechnic University. All subjects were fully informed of the possible risks and gave written, voluntary consent.

Follow up visits

Complete ophthalmic examinations with visual field, OCT and the mfERG recordings were scheduled every 12 months over a four-year period. After the first examination, six subjects withdrew, and at subsequent visits, none, three and one further subjects withdrew. Eighteen subjects completed all the examinations, including eight patients with asymmetric glaucoma (8 eyes) and 10 subjects with ocular hypertension (20 eyes).

Multifocal ERG stimulation and recording:

The mfERG program (VERIS 4.1; EDI, San Mateo, CA) was run on a Macintosh G3 computer (Apple Computer, Cupertino, CA) and the mfERG stimulus pattern was presented on a 19-inch RGB monitor (model GDM-500PS; Sony, Tokyo,

Japan). The mfERG was measured using the luminance-modulated global flash mfERG paradigm.[23] In this paradigm, each m-sequence stimulation cycle consisted of four video frames; each frame lasts 13.3 ms when a 75 Hz frame rate is used. There was an initial multifocal pattern with 103 hexagons, scaled with eccentricity, and each hexagon was either bright or dark according to a pseudorandom binary m-sequence (2^{13} –1). After the multifocal pattern, there was a dark frame (0.04 cd·s/m^2, i.e. 3 cd/m^2 per frame), a full screen global flash (2.16 cd·s/m^2, i.e. 162 cd/m^2 per frame), and a second dark frame before the next multifocal pattern stimulation. The average luminance of the multifocal patterns was about 1.11 cd·s/m^2 (i.e. 83 cd/m^2 per frame) and the background of the stimulation was 83 cd/m^2. Four different stimulus-contrast conditions under the global flash paradigm were used and the luminance-difference of the multifocal patterns were set at 2.12, 1.42, 1.08 and 0.62 cd·s/m^2 (Figure 1). The presentation order of the four stimulus conditions was randomised across subjects.

A Dawson-Trick-Litzkow (DTL) electrode was used as the active electrode and gold-cup surface electrodes were used for both reference and ground. Before testing, the pupil of the tested eye was fully dilated to at least 7 mm diameter, with 1% Tropicamide (Alcon). During the mfERG recording, the refractive error of the tested eye was fully corrected for the viewing distance of 30 cm. The size of the stimulation pattern was 42^o vertically and 48^o horizontally. The signal was amplified using a

Grass P511K amplifier (bandpass: 10 to 300Hz; gain: x100,000). The subjects would fixate at the central cross in the stimulation pattern during the measurement. The recording was monitored using the real time signals shown by the VERIS program; any recording segments contaminated with blinks or small eye movements were rejected and immediately re-recorded. Recordings were divided into 16 slightly overlapping recording segments and the recording time for each stimulation cycle was approximately 8 minutes.

Data analysis

Only data from eyes with measurements from all five visits were used for analysis. Peripheral mfERG responses (19^o – 48^o) were of interest in this study because glaucomatous visual field defects initially occur in the Bjerrum area. Based on our recent studies,[22, 23] responses from the three peripheral rings of the mfERG responses were grouped in quadrants (Figure 2a). These responses have similarities in waveform and latency, as well as similar characteristics in the luminance-modulated response function. The mfERG findings in different quadrants of the field were represented by calculating the adaptive index where the DC responses from different stimulus conditions were plotted as a function of the luminance difference value of the stimulus. The way in which these functions varied over time in the subjects with

high risk of glaucoma were indicated by the adaptive index value, reflecting the degree of saturation of the DC luminance-modulated response.[22, 23]

To evaluate the co-variation of the adaptive index associated with glaucoma, the corresponding field regions with abnormal (lower than 1.5) and normal (more than 1.5) initial adaptive indices [23] were grouped separately to assess the changes of visual field test and OCT over the course of the study. The relationship between the peripheral mfERG response and the visual field mean defect was evaluated by comparing the measurements averaged within each quadrant. In order to compare with the OCT values, the mfERG responses were regrouped in an arcuate area [29] and compared with the corresponding sectors of the OCT results (Figure 2b).

As well as using eyes with asymmetric glaucoma, both eyes from the ocular hypertension subjects were analyzed. The statistical method of generalized estimating equations (GEEs) in SPSS 16.0 was applied to account for the possible correlation of measurements from both eyes of the same subjects and from multiple quadrants of the same eye.[30] The GEE method allows for working with correlated data, and estimates models that account for the correlation; these models correct the variance-covariance matrix on a weighted scale. It thus can help to analyse dependent data and can account for related measurements from left and right eyes of the same patient, where highly correlated data have been scaled as less influence on the final results. The 0.05 level

was used as the criterion level for statistical significance in all tests and Bonferroni *post-hoc* test was used for analysis of repeated measurements.

Results

The fellow eyes from asymmetric glaucoma subjects had low values of the adaptive index with a mean of 0.26 ± 1.46 in the first visit, while eyes with ocular hypertension had a mean value of 1.69 ± 1.92 (GEE: p = 0.002) (Figure 3). Even though different values of adaptive index were shown between groups, and the adaptive index of the fellow eyes from asymmetric glaucoma subjects are known to be compromised,[22] the visual field and the OCT results were still clinically normal (within the age-matched normal limits) in all subjects. Among the subjects in these two groups, some of the field quadrants already had an abnormal value of adaptive index (below 1.5) as defined in our previous study.[23] There were abnormal adaptive indices in 27 of 32 field quadrants (84 %) in asymmetric glaucoma subjects, and abnormal adaptive indices in 38 of 80 field quadrants (48 %) in subjects with ocular hypertension.

Since the two high risk groups are likely to differ in their progress for developing glaucoma, they were analysed separately. Quadrants in the field with adaptive index below 1.5 in the first visit were considered as abnormal.[22, 23] In subjects with asymmetric glaucoma, over 80% of field quadrants had abnormal initial adaptive index (-0.15 ± 1.17), and there was a significant reduction of the adaptive index to -1.04 ± 1.07 over the period of this study (GEE with *post hoc* Bonferroni: p < 0.001)

(Figure 4a). The field quadrants with initially normal adaptive index in these patients (2.46 ± 0.56), showed no significant reduction of value (GEE with *post hoc* Bonferroni: p = 0.458). For the patients with ocular hypertension, the field quadrants with initially normal index had a mean value of 3.16 ± 1.29; the field quadrants with initially abnormal index had a mean value of 0.07 ± 0.93. There was no significant reduction of adaptive index for any field quadrants in these patients (GEE with *post hoc* Bonferroni: p = 0.195 and p = 0.793, respectively) (Figure 4b).

In order to observe the changes of visual sensitivity in the field corresponding to the mfERG response, the visual field data from each high risk group was divided into two categories according to whether the initial adaptive index was above or below 1.5. Generally, the visual fields in the category with an initially abnormal adaptive index (below 1.5) had lower mean defects than those with a normal adaptive index. Subjects with asymmetric glaucoma and an abnormal adaptive index had an initial mean defect of -2.04 ± 1.70 dB with a reduction rate of -0.17 dB/year in corresponding field quadrants (Figure 5a). For subjects with ocular hypertension, the mean defect for quadrants with an initial abnormal adaptive index was -1.19 ± 1.17 dB, with a reduction rate of -0.10 dB/year (Figure 5b). Neither group showed a significant reduction of the visual field mean defect over time when compared with the initial visual field mean defect despite the adaptive index for these field quadrants

being in the abnormal range (GEE with *post hoc* Bonferroni: p = 0.338 and p = 0.353, respectively).

Since the OCT values represent sectoral regions of the optic disc, rather than field quadrants, mfERG responses were regrouped into superior and inferior arcuate regions according to the arrangement of the OCT setting (see Figure 1b). Arcuate regions with adaptive index above and below 1.5 in the first visit were extracted separately for comparison with the OCT data. As with the visual field results, the retinal nerve fiber layer was generally thinner in those regions with abnormal adaptive index than in those with normal adaptive index. Subjects with asymmetric glaucoma (Figure 6a), who initially had an abnormal adaptive index, showed thinner nerve fiber layers (122.57 ± 25.74 μm) than the ocular hypertension groups (133.50 ± 26.49 μm) (Figure 6b). A significant thinning of the retinal nerve fiber layer was found over the period of this study with a reduction rate of -3.69 μm/year in the asymmetric glaucoma group (GEE with *post hoc* Bonferroni: $p < 0.001$) and -3.59 μm/year in the ocular hypertension group (GEE with *post hoc* Bonferroni: p = 0.01). In contrast, there was no significant thinning of the retinal nerve fiber layer thickness over time in the arcuate region which initially had a normal adaptive index (GEE with *post hoc* Bonferroni: p = 0.583 and p = 0.258, respectively) .

In the last visit, two subjects from the asymmetric glaucoma group developed manifest glaucomatous damage. A glaucomatous visual field defect (inferior nasal) was found in subject 8 (Figure 7a); this quadrant had shown an abnormal adaptive index at the first visit. There were three quadrants with abnormal adaptive indices in the mfERG measurement at the first visit, and the lowest value was found in the inferior nasal quadrant. Although the adaptive index for the four quadrants showed a reduction over time, the quadrant with the lowest initial adaptive index was the first to develop a field defect. A reduction of retinal nerve fiber layer thickness (outside normal limit) was found in the inferior region of the retina of subject 2 (Figure 7b); the corresponding region had an abnormal adaptive index at the first visit. At the first visit, both the superior and the inferior region showed an abnormal adaptive index, but the lowest value was found at the inferior retinal region, which developed the clinically reduced retinal nerve fiber layer thickness first over the four years of the study.

Discussion

Different initial adaptive indices were found between groups in this study, but subjects recruited in different groups may be at different points of the timeline of glaucoma progression, and has the potential to complicate interpretation, making it difficult to decide which group is more likely to develop glaucoma. However, the fact that some field quadrants initially had abnormal adaptive index values (less than 1.5) shows that some of our subjects already have functional changes in the retina, even though no abnormities were revealed by visual field or OCT measures. The global flash paradigm with luminance modulation is designed to measure retinal adaptive changes.[22, 23] We have reported the way to quantify this response function by calculating the adaptive index, this index is significantly reduced in glaucoma subjects and is well correlated with glaucomatous visual field defects.[23] Moreover, our previous study has shown that the best cutoff point of the adaptive index for glaucoma differentiation is 1.5, which optimized the sensitivity/specificity relationship, giving sensitivity of 93% and specificity of 95%.[23]

Subjects with unilateral glaucoma show impairment of the fast-adaptive mechanism before any visual field defect becomes apparent in the fellow eye,[22] which initially has an abnormal adaptive index. In addition, subjects with ocular hypertension were also shown to have abnormal mfERG responses [16]; the pattern

ERG measurement has also been reported to predict progression to glaucoma at least one year prior to conversion.[31] The current study shows that subjects with asymmetric glaucoma have lower adaptive indices in their fellow eyes. These eyes have a higher percentage of field quadrants with an initially abnormal adaptive index than the eyes of ocular hypertension subjects.

POAG is a chronic eye disease with slowly progressive loss of retinal ganglion cells. In order to investigate the co-variation of the adaptive index in retinal functional/structural changes for subsets of subjects at high risk of developing glaucoma damage, it would be desirable to observe the progression of glaucomatous changes. Thus the field quadrants with the initially obtained normal or abnormal adaptive index were compared in the longitudinal examination of visual field and OCT results in order to focus on the functional and structural changes of those particular field regions. .

Generally, regions with abnormal adaptive index had lower visual field mean defect and thinner retinal nerve fibre thickness than those with normal adaptive index. But, in this study, there was no significant reduction in the visual field sensitivity over the study period although the fast-adaptive mechanism was initially impaired. This may relate to the relative poor sensitivity of perimetry in detecting visual field changes in the early stages of glaucoma.[11] Although static white-on-white threshold

perimetry, which shows reduction of visual sensitivity as a result of retinal ganglion cell loss, has become the standard procedure for assessment of glaucoma,[10, 32] a substantial degree of ganglion cell loss must occur before standard perimetry shows significant visual field defects.[11, 33, 34] Indeed, the percentage of progression to glaucomatous visual field loss five years after initial diagnosis is only 7.2 % of the fellow eyes in patients with unilateral POAG.[35] Other studies have reported that about 24 – 43 % of fellow eyes in patients with unilateral POAG develop glaucomatous visual field loss over a 3 to 7 years period.[2-5] In addition, the conversion rate from untreated ocular hypertension to glaucoma is only about 1 % per year and the conversion rate possibly decreases to half for treated ocular hypertension.[8] Over our four-year observation period, only one subject with asymmetric glaucoma developed a glaucomatous visual field defect in the fellow eye. The field defect was developed first in the quadrant with the lowest initial adaptive index.

The OCT provides an *in-vivo* objective measurement of the peripapillary retinal nerve fiber layer thickness. The retinal nerve fiber layer thickness measured by the OCT has been demonstrated to have high diagnostic value in early glaucoma,[36-40] and provides higher sensitivity than conventional visual field testing.[41] A loss of 10 µm in retinal nerve fiber layer thickness from baseline has been shown to predict glaucomatous change.[42] Moreover, the association between OCT findings and visual

field loss is strong [38, 43] with good reproducibility in both normal and glaucomatous eyes.[44, 45] All of these findings clearly demonstrate that OCT can be used in the diagnosis and monitoring of glaucoma, but only indicates disease after ganglion cells have been lost.

In relating retinal function in visual field sectors to structure of optic nerve head sectors, arcuate nerve fiber bundles in Bjerrum's area were chosen for analysis because the arcuate fibers entering the optic nerve head at the 12 and 6 o'clock sectors are most sensitive for glaucoma differentiation.[39] Thus the mfERG responses in this study were regrouped in the same regions for comparison with the corresponding regions of the OCT results. When the adaptive index was initially abnormal, significant reductions of the retinal nerve fiber layer thickness were observed over the study period in both the asymmetric glaucoma and ocular hypertension groups. We have made an assumption that an adaptive index value of 1.5 would discriminate in this region of the visual field, as it does for field quadrants,[22, 23] but this assumption should be tested in further experiments. Thinning of the nerve fiber layer is one of the indicators of glaucomatous changes.[42] The retinal nerve fiber layer thins with increasing age, but the loss of retinal layer thickness with age is only 3.3 µm per decade,[46] which is only one tenth of the loss observed in this study (-3.69 and -3.60 µm/year for the asymmetric glaucoma and ocular hypertension group, respectively).

However, reductions in retinal thickness were not observed in regions with initially normal adaptive indices. Hence, the thinning of the retinal nerve fiber layer indicates progression of glaucoma, and this further supports the hypothesis that early retinal nerve fiber loss shown in the OCT precedes visual field defects shown in standard perimetry.[41] Furthermore, since the adaptive index is based on interaction of local and global flashes and represents the retinal fast-adaptive mechanism, the abnormal index appears to support the estimation of increased glaucoma risk and the fast-adaptive mechanism seems to be impaired before structural changes can be measured or observed in the retina. With the retinal nerve fiber layer beginning to reduce in thickness, both ocular hypertension and asymmetric glaucoma groups in this study seem to have an increased risk of developing glaucoma. Besides the subject who developed glaucomatous visual field defects, another asymmetric glaucoma subject also developed an abnormal retinal nerve fiber layer thickness over the study period. The field showed an abnormal initial adaptive index before the abnormal retinal nerve layer thickness was found, and this suggests that the change in the fast-adaptive mechanism occurs at least 3 to 4 years before the structural changes in the retina.

In conclusion, the adaptive index derived from the modified global flash mfERG paradigm has demonstrated good sensitivity for detecting subjects with real risk of glaucoma development and may assist in predicting progression of glaucoma. We

believe that the modified global flash mfERG paradigm could be used for early detection of glaucoma in a wider ranging prospective clinical trial of patients who show signs but fail to reach the threshold for a clear diagnosis of glaucoma.

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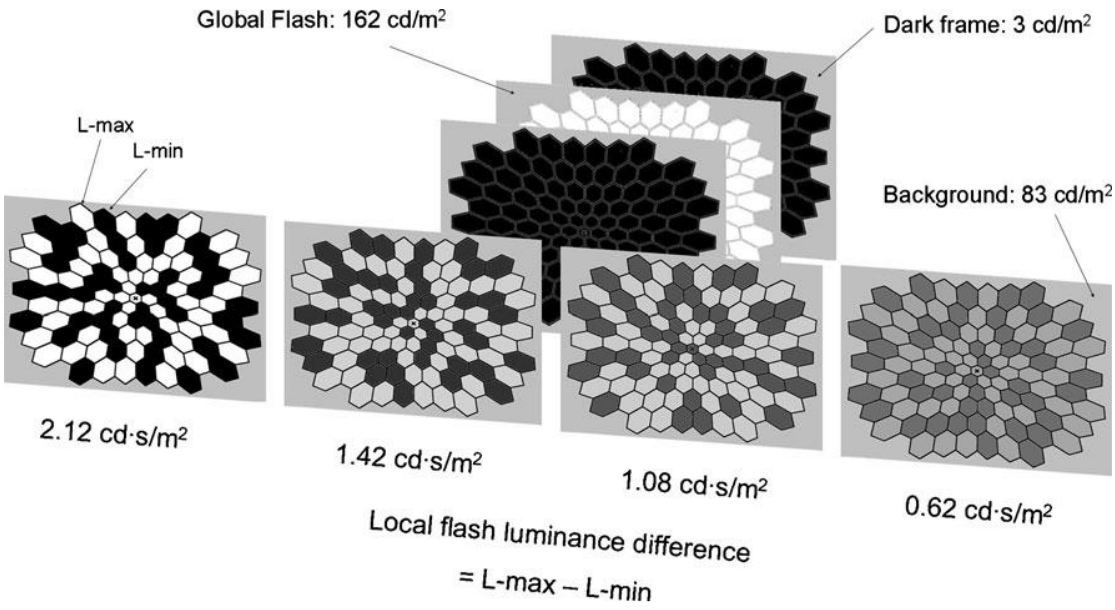

Figure 1 The stimulus sequence contains 4 frames. The initial frame (multifocal flash) alternated between bright and dark according to a pseudo-random binary m-sequence with a preset stimulus-contrast level. The luminance differences between the brighter hexagons and the dimmer hexagons (L-max – L-min) of the multifocal flashes in the four stimulus-contrast settings are denoted 2.12 cd·s/m^2, 1.42 cd·s/m^2, 1.08 cd·s/m^2 and 0.62 cd·s/m^2; this frame was followed by a dark frame (0.04 cd·s/m^2). A global flash (2.16 cd·s/m^2) (frame 3) was then presented, followed by a second dark frame (0.04 cd·s/m^2).

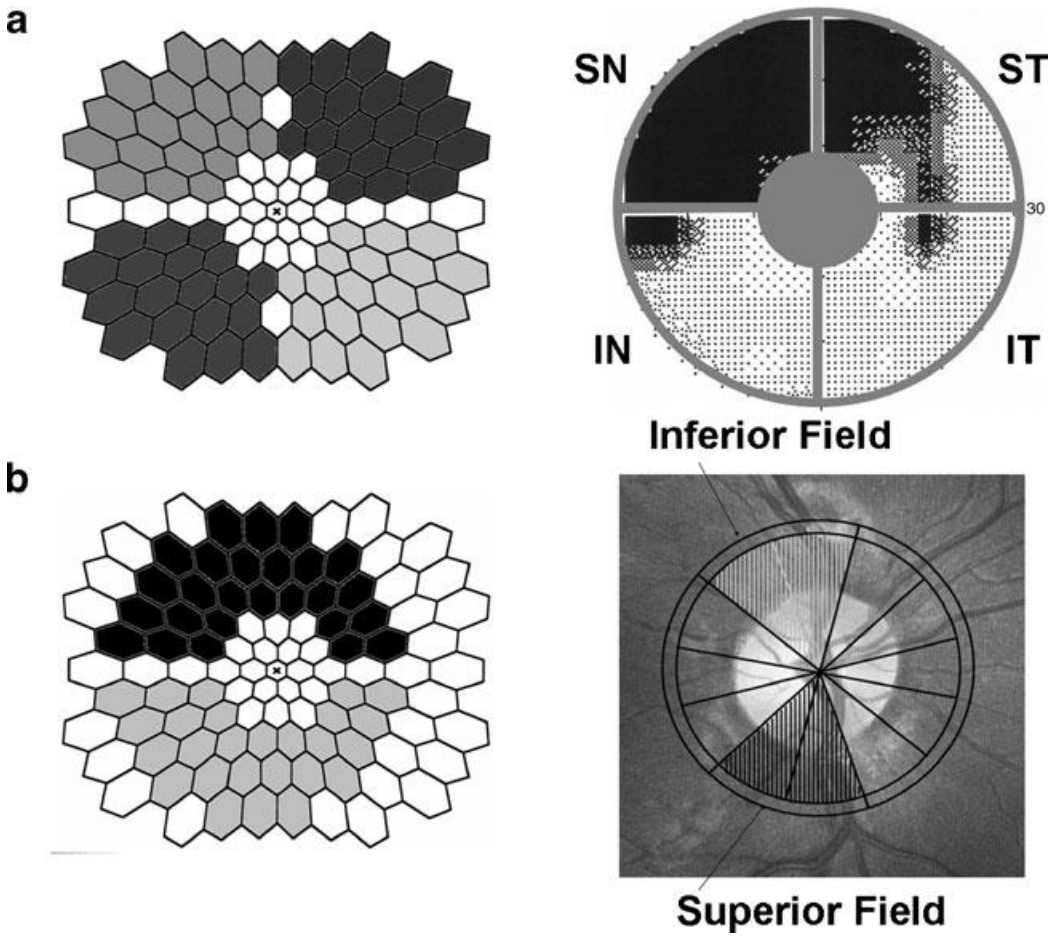

Figure 2 **(a)** The global flash mfERG responses from the peripheral rings were grouped as peripheral responses, which were further averaged into the visual field quadrants shown here. **(b)** The peripheral global flash mfERG responses were divided into superior and inferior field for comparison with the corresponding optic nerve head sectors from the OCT assessment.

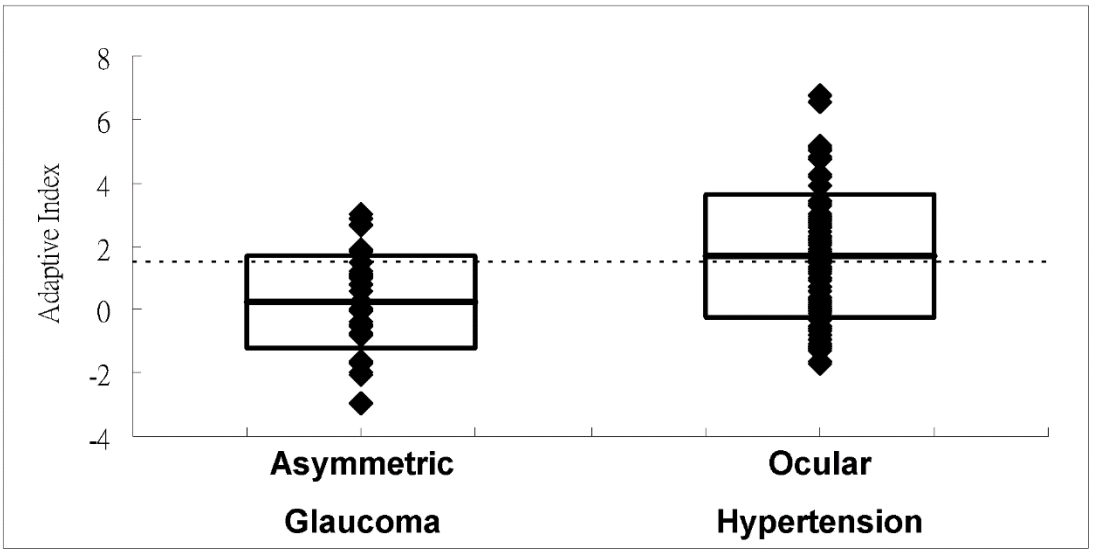

Figure 3 The adaptive index for field quadrants of the two high risk groups at the first visit. Dotted line: best cutoff point of the adaptive index (1.5) for glaucoma differentiation; middle line: the mean; top and bottom box edges: ±1 SD. (♦) The individual quadrant values from subjects in the two high risk groups.

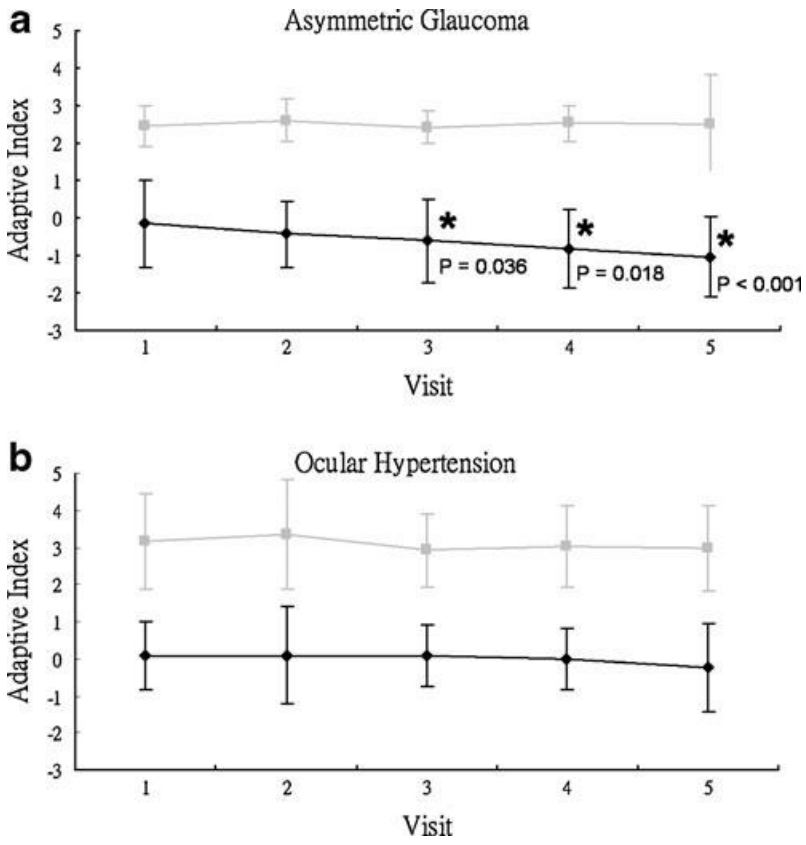

Figure 4 Adaptive index for field quadrants for subjects in the two high risk groups **(a)** asymmetric glaucoma and **(b)** ocular hypertension. Initially normal adaptive index (grey) and initially abnormal adaptive index (black). Bars indicate standard deviation. Results marked with (*) showed significant reduction at the levels shown.

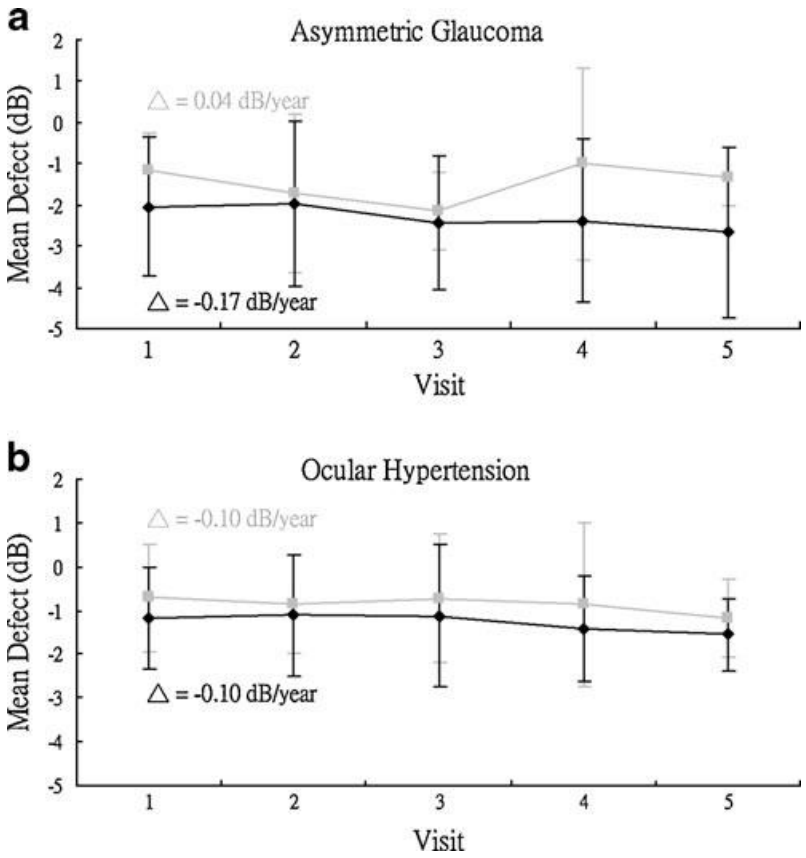

Figure 5 Visual field mean defect from subjects in the two high risk groups **(a)** asymmetric glaucoma and **(b)** ocular hypertension, divided into two groups based on the initial adaptive index value. The mean defect with initially abnormal adaptive index value (black) and those with initially normal adaptive index (grey) showed no significant changes over the course of the study. Bars indicate standard deviation and △ indicates rate of change.

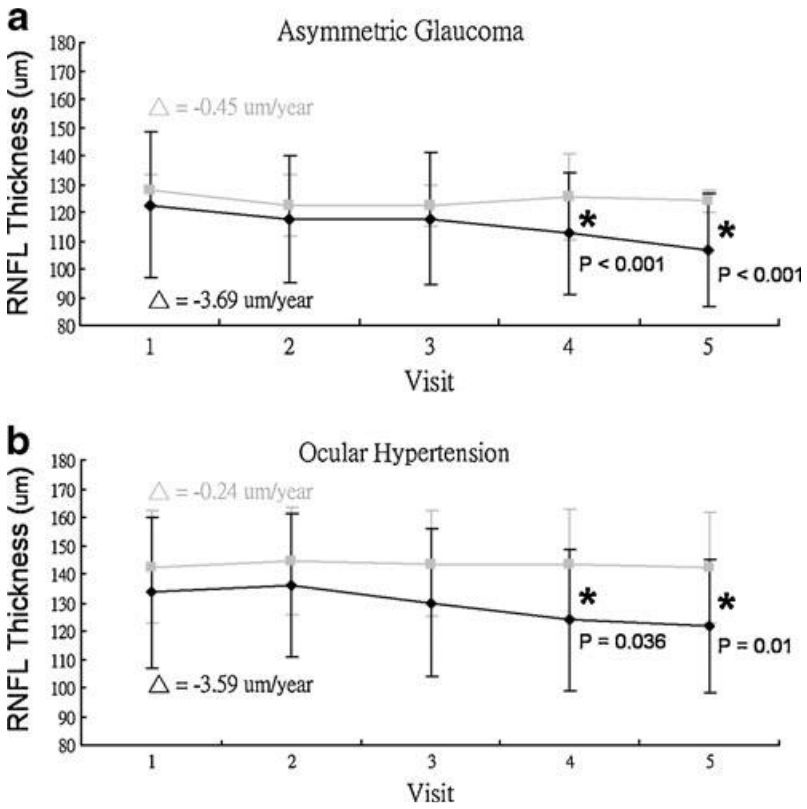

Figure 6 Retinal nerve fiber layer thickness in appropriate optic disc sectors measured by OCT from subjects with **(a)** asymmetric glaucoma and **(b)** ocular hypertension divided into two groups based on the initial adaptive index value. Retinal nerve fiber layer thickness with abnormal initial adaptive index value (black) showed significant thinning (*) at the levels shown for the last two visits in subjects with abnormal initial adaptive index for asymmetric glaucoma and ocular hypertension. Retinal nerve fiber layer thickness was unchanged for those with initially normal adaptive index value (grey). Bars indicate standard deviation and △ indicates rate of change.

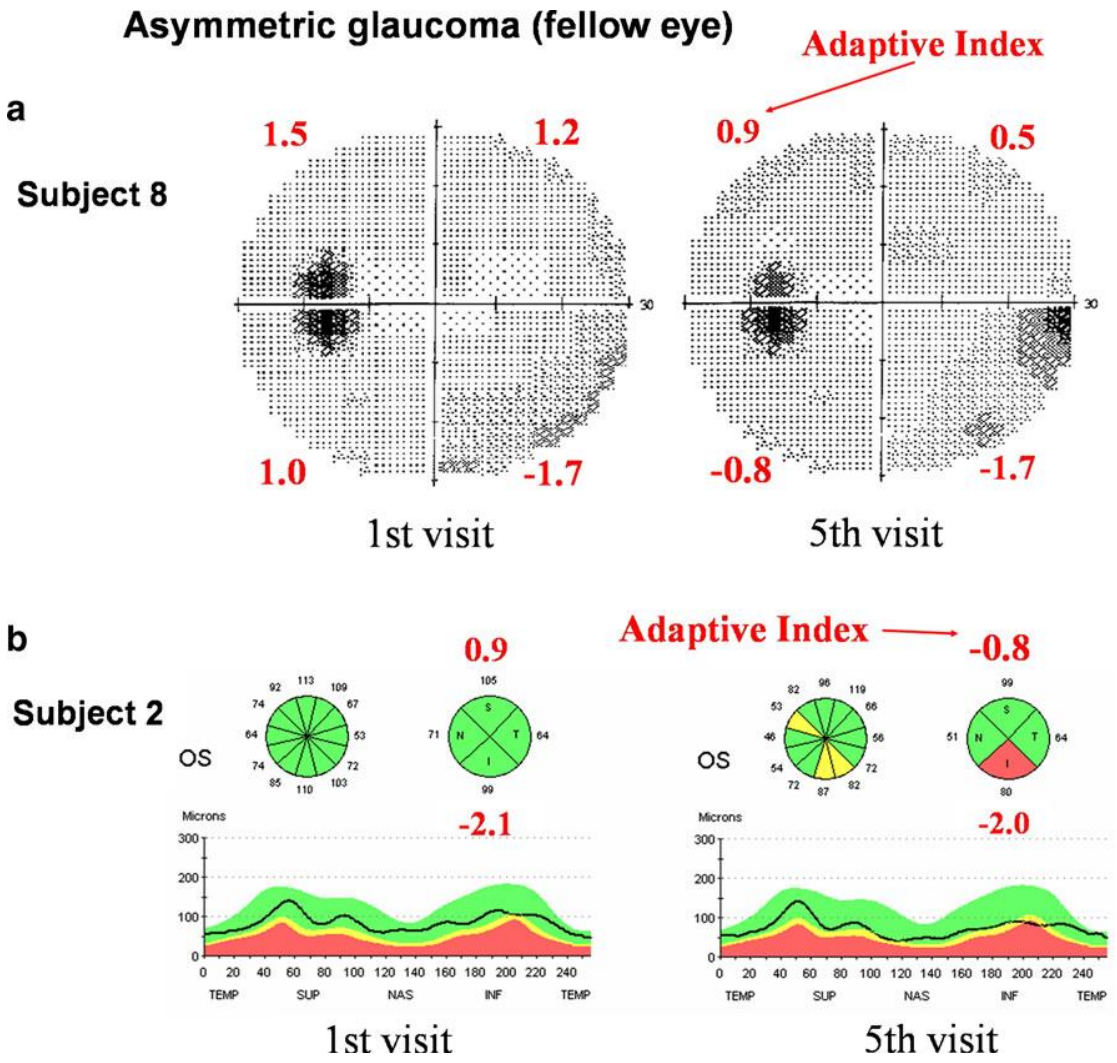

Figure 7 The clinical data from two of the asymmetric glaucoma subjects who developed manifest glaucomatous changes in visual field and/or OCT during the study period (see text).