Correlation Between En Face Optical Coherence Tomography Defects of the Inner Retinal Layers and Ganglion Cell Inner Plexiform Layer Analysis After Internal Limiting Membrane Peeling for Idiopathic Full-Thickness Macular Hole

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PURPOSE. The purpose of this study was to report en face optical coherence tomography (OCT) inner retinal changes after internal limiting membrane (ILM) peeling for idiopathic full-thickness macular hole (IFTMH) and to correlate these findings with macular ganglion cell inner plexiform layer (GC-IPL) analysis.

METHODS. This prospective study included 20 patients with IFTMH treated using pars plana vitrectomy with ILM peeling. All patients were analyzed using en face OCT at 6 months after surgery to determine the effect of ILM peeling on the inner retinal layers. Correlation between the GC-IPL en face OCT findings and that obtained by three-dimensional volumetric OCT scanning also was performed.

RESULTS. Seven patients (35%) showed defects in the retinal nerve fiber layer (RNFL) that appeared as multiple dark dots with no visible defects at the GC-IPL, either with en face OCT or 3D volumetric OCT scanning. Thirteen patients (65%) showed a similar combination of RNFL defects and well-circumscribed defects in the underlying GC-IPL. These defects could be visualized on en face OCT display, and they correlated with areas of GC-IPL thinning detected in the 3D volumetric OCT scanning.

CONCLUSIONS. With ILM peeling, en face OCT scanning showed two forms of inner retinal layers changes. The first form was the concentric macular dark spots (CMDS) with intact GC-IPL. The second form appeared in the CMDS with evident localized defects in the underlying GC-IPL. These defects correlate with the areas of GC-IPL thinning detected using 3D volumetric OCT scanning.

Keywords: en face OCT, ganglion cell inner plexiform layer, internal limiting membrane peeling

Since the introduction of pars plana vitrectomy (PPV) for closure of idiopathic full-thickness macular hole (IFTMH) by Kelly and Wendel in 1991, there have been several refinements in surgical technique aimed at improving anatomical and functional outcomes after surgery.1-3 The rate of anatomical closure of IFTMH improved significantly after internal limiting membrane (ILM) peeling.4-7 Regardless of its beneficial effects, ILM peeling has also been shown to lead to some structural and functional effects. These effects include: damage to Müller cells, because the ILM corresponds to their basal membrane, delay in the recovery of the b-waves of focal macular electroretinograms8 and decrease in thickness of the macular retinal layers.9

Several studies have reported the effect of ILM peeling on the inner layers of the retina. Previous studies reported reduction of thickness of ganglion cell-inner plexiform layer (GC-IPL) after ILM peeling during PPV for IFTMH.10,11 In addition, an appearance referred to as dissociated optic nerve fiber layer (DONFL) was reportedly observed by fundus photography after ILM peeling.12,13 The DONFL appearance was described as arcuate slightly dark striae within the posterior pole along the course of optic nerve fiber.

Previous studies using optical coherence tomography (OCT) have focused mainly on the predictive factors for MH surgery.14-22 Also, studies have correlated the surgical outcome with integrity of the outer retinal layers, especially the photoreceptors.23-25 The advancement of spectral-domain OCT (SD-OCT) combined with the development of intraretinal boundary segmentation algorithm has enabled more selective assessment of different layers of the macula. These advances include the ability to automatically measure retinal nerve fiber layer (RNFL) and GC-IPL.26-29 In addition, advances have helped in the development of en face OCT. En face OCT is an imaging modality that facilitates the assessment of retinal structures transversely and allows layer-by-layer retinal visualization.30-33 Alkabes et al.34 examined the appearance of DONFL using en face SD-OCT and observed multiple dark dots along the course of RNFL. They named this appearance concentric macular dark spots (CMDS).
The aim of this study was to report en face OCT inner retinal changes after ILM peeling for IFTMH and to correlate these findings with macular GC-IPL analysis.

**PATIENTS AND METHODS**

This nonrandomized prospective study consisted of 20 consecutive patients with IFTMH. All surgical procedures took place at Mansoura Ophthalmic Center between January 2014 and December 2014.

Inclusion criteria were patients who had successfully undergone PPV with ILM peeling for an IFTMH (larger than 400 μm), with postoperative follow-up period of at least 6 months. Patients were excluded if they had high myopia (≥6 D or axial length [AL] ≥26 mm), eyes with postoperative complications, such as reopening of the MH, a history of ocular surgeries or trauma, and patients with ocular or systemic disorders which could affect the inner RNFL and GC-IPL (e.g., glaucoma, age-related macular degeneration, or optic nerve diseases). Patients with optical media opacity that significantly disturbed OCT image acquisition (e.g., significant postoperative cataract or posterior capsular opacity in either eye) were also excluded. The study protocol was approved by the Institutional Review Board of Mansoura Ophthalmic Center and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients after the nature of the study was explained to them in clear understandable words.

All patients underwent comprehensive ophthalmologic examinations including measurement of best-corrected visual acuity (BCVA), IOP by Goldmann applanation tonometry, noncycloplegic refraction (autorefractor KR-8900; Topcon Corporation, Tokyo, Japan), AL (Nidek Echoscan-2000, Birmingham, UK), slit-lamp biomicroscopy; indirect fundus examination, and SD-OCT (2000, Topcon Corp.). Best-corrected visual acuity (BCVA), IOP by Goldmann applanation tonometry, anterior segment examination including measurement of best-corrected visual acuity, IOP by Goldmann applanation tonometry, and noncycloplegic refraction were performed before surgery and 6 months after surgery, using Topcon 3D OCT-2000 version 8.20 (Topcon Corp.). Spectral-domain optical coherence tomography assessment included a three-dimensional (3D) macular cube (6.0 × 6.0 mm–512 × 128) and a 3D (V) macula scanning (7.0 × 7.0 mm–512 × 128). In addition, a five-line cross-scan across the hole was performed for horizontal and vertical images. An internal fixation target was used to improve reproducibility, but if fixation was poor, an external fixation target was used instead. Pupil dilation with tropicamide 1% and phenylephrine 2.5% was done prior to scanning. Scans were performed at least twice to ensure good quality scan acquisition. They were defined as scans with image quality of 40 or more (default good image quality is 30), without discontinuity or misalignment, involuntary saccade or blinking artifacts, and absence of algorithm segmentation failure on careful visual inspection.

The 3D macular cube was used to create the en face images. Several layers could be selected as the base layer, namely ILM, photoreceptors inner/outer segment (IS/OS), retinal pigment epithelium, and Bruch’s membrane. After selecting the ILM as a reference layer, image was flattened for best visualization of the inner retinal layers. Line scans were manually adjusted both horizontally and vertically and then moved deeply into the retinal layer in order to obtain the desired enface image for the ILM, RNFL, and GC-IPL.

The five-line cross report was performed across the center of the hole. Its averaging and overlapping allowed excellent visualization of different layers. It was used (in conjunction with the 3D scan) to ensure closure of the hole, restoration of the IS/OS integrity, external limiting membrane, and normal foveal contour.

Macular ganglion cell layer (GCL) assessment was performed by using the 3D volumetric OCT scanning: scan pattern 3D (V), scan length 7.0 × 7.0 mm, scan resolution 512 × 128 pixels, and fixation macula. The cube consists of 50,000 A-scans per second centered on the fovea. Report print-outs (Fig. 1E) include color fundus image and vertical cross-section line scan (first row), thickness map (second row), significance map (third row), average thickness, and asymmetry map (fourth row), which show three parameters referring, from left to right, to RNFL thickness, GCL+ (corresponding to GC-IPL thickness), and GCL++ (corresponding to RNFL/GC-IPL thickness), respectively. Only the GC-IPL parameter was used in this study for statistical analysis.

**Main Outcome Measures**

The main outcomes were to report the rate of en face OCT of the late inner retinal changes after ILM peeling for IFTMH and to correlate between these findings and the macular GC-IPL analysis.

**Statistical Analysis**

Data were analyzed using SPSS version 20 software (SPSS; IBM, Armonk, NY, USA) for Windows (Microsoft, Redmond, WA, USA). Quantitative variables with normal distribution were described as mean (±SD). Independent t test was used to compare mean ganglion cell layer thickness values in the same eye before and after surgery. Wilcoxon signed rank test was used to compare visual acuity in the same eye before and after surgery. A P value of ≤0.05 was considered statistically significant.

**RESULTS**

Twenty eyes of 20 patients who had successful PPV and ILM peeling for IFTMH were included in this prospective study. The mean age was 63.5 ± 2.417 years (range, 60–68 years), and
there were 11 females (55%) and 9 males (45%). The operated eye was the right eye in 9 patients (45%) and left eye in 11 patients (55%). Mean preoperative BCVA was 0.99 ± 0.18 logMAR units and 0.47 ± 0.16 logMAR units postoperatively. Improvement in BCVA from baseline was statistically significant (P ≤ 0.001). Patient demographic and clinical data are summarized in Table 1.

Six patients (30%) were pseudophakic, and eight patients (40%) underwent phacoemulsification with foldable lens implantation during surgery. No clinically significant cataract was observed in the remaining phakic eyes during OCT scanning.

The mean AL was 22.97 ± 0.85 mm (range, 20.5–23.75 mm) and that of the refractive error was −1.52 ± 0.64 D (range, −0.75 to −2.25 D). There were no significant differences between preoperative and postoperative IOP and OCT image quality (P = 0.667 and 0.632, respectively). Mean ± SD postoperative follow-up period was 9.4 ± 0.87 months (range, 6–11 months).

Table 2 shows individual preoperative and postoperative superior, inferior, and total macular GC-IPL thickness (in micrometers). The preoperative and postoperative comparison of total, superior, and inferior GC-IPL thickness is shown in Table 3. Significant thinning of the total, superior, and inferior GC-IPL was detected postoperatively, but it was more evident superiorly.

None of the patients showed defects in RNFL preoperatively. Defects in RNFL were evident in all patients (100%) postoperatively. Defects were found along the course of retinal nerve fibers in the area of ILM peeling.
En Face OCT Inner Retinal Changes After ILM Peeling

### TABLE 2. Preoperative, Postoperative, and Total Macular GC-IPL Thickness

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<tr>
<th>Patient</th>
<th>Preoperative Thickness</th>
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<td>Superior, Inferior, Total</td>
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GC-IPL, ganglion cell inner plexiform layer.

En face OCT scanning at the level of the GC-IPL showed two groups of patients. Seven patients (35%) (Table 2, patients 1–7) showed no visible defects at the GC-IPL postoperatively. In these patients, GC-IPL analysis of the 3D volumetric OCT scanning showed intact GC-IPL with normal and nearly unchanged thickness, and defects in the RNFL appeared as multiple dark dots along the course of retinal nerve fibers in the area of the ILM peeling resembling the CMDS described by Alkabes.34 Figure 1 shows the preoperative and postoperative en face OCT scanning at two levels: RNFL with visible postoperative CMDS and deeper scans at the level of GC-IPL with no visible defects (Table 2, patient 1).

The second group consisted of 13 patients (65%) (Table 2, patients 8–20). In this group well circumscribed defects at the level of the GC-IPL were detected. All the defects were located superiorly and superiotemporally. When we compared these defects with GC-IPL analysis of the 3D volumetric OCT scanning, they correlated both in location and extent with the areas of thinning. Also, in this group, the CMDS were evident at the level of the RNFL. Figures 2 and 3 show preoperative and postoperative en face OCT scanning with visible postoperative CMDS at the level of RNFL, whereas deeper scans at the level of GC-IPL showed well-circumscribed defects that correlated with areas of thinning seen with GC-IPL analysis of the 3D volumetric OCT scanning (Fig. 2, patient 10; Fig. 3, patient 20; Table 2).

### DISCUSSION

En face OCT scanning provides cross-sectional images of retinal layers that bear a strong resemblance to histology. This enables visualization of the lateral extent of structures which is particularly important in such pathologic conditions affecting different retinal layers.35

Recent advances in OCT technology have enabled a more precise structural assessment of the macular region. For example, GC-IPL analysis was developed as an additional tool for assessing structural change in glaucoma.36–38

In recent years, ILM peeling became a common step during MH surgery as it increased the macular hole closure rate, especially in stages III and VI.39,40 Although there were some initial concerns regarding the possibility of macular trauma from ILM peeling based on persistent electoretinography abnormalities and toxicity of some of the dyes used to stain the ILM, all these seemed not to influence the visual outcomes after MH surgery with ILM peeling. However, histologic studies have shown that surgically removed ILM specimens from eyes with MH or pucker contain neuronal elements. These findings raised concerns of damage to inner retinal tissue during ILM peeling.11

Tadayoni et al.13 were the first to document changes occurring in the inner retina after ILM peeling by using blue-filtered fundus photographs. They found arcuate, slightly dark striae within the posterior pole along the course of optic nerve fiber, which they referred to as DONFL. Alkabes et al.34 retrospectively reviewed 36 cases of IFTMH surgery with ILM peeling using en face SD-OCT images to report the DONFL appearance. In that study, this feature was evident on OCT scans in 100% of patients at 3 months after surgery. Based on its appearance, they named this feature CMDS. In these previous studies, the changes described were similar and were detected in the RNFL after ILM peeling.

We performed our study in patients who had successfully undergone PPV with ILM peeling for a unilateral IFTMH with postoperative follow-up period of at least 6 months. The aim was to detect changes in the inner retinal layers by en face OCT imaging and to correlate these findings with those obtained by 3D volumetric OCT scanning.

Our study was not designed to assess the value of ILM peeling in MH surgery. Therefore, only patients with anatomically successful IFTMH surgery were included. Any case that required reopening of the hole was considered unsuccessful surgery and was excluded from the study. According to Kang et al.,41 the closed macular holes were divided into two patterns based on OCT, type I closure, that is, closed without foveal neurosensory retinal defect, and type 2 closure, that is, closed with foveal neurosensory retinal defect. In our study, 18 eyes (90%) showed type 1 closure, and 2 eyes (10%) showed type 2 closure.

Two different forms of postoperative inner retinal layers changes were observed in this study. The first form showed only CMDS at the level of RNFL with no evidence of GC-IPL defects on en face OCT scanning or evident thinning on 3D volumetric OCT scanning. This appearance of CMDS is similar to that reported by previous studies.34,42 The second form showed a combination of CMDS at the level of the RNFL and well-circumscribed defects located at the level of the GC-IPL. Ganglion cell-inner plexiform layer defects were detected superiorly and temporally in 65% of cases after surgery. Ganglion cell-inner plexiform layer defects can be visualized on en face OCT display and correlate well with areas of thinning on the GC-IPL thickness maps derived from 3D volumetric OCT scanning. To our knowledge, we are the first to report en face OCT defects of GC-IPL after ILM peeling for IFTMH.
Our results suggest that, with ILM peeling, two forms of inner retinal layers changes occur. The first form is CMDS with intact GC-IPL. The second form is a combination of CMDS at the level of the ILM combined with localized defects in the underlying GC-IPL.

The thickness provided by the software was automatically compared by the device to its normative database; any abnormality was displayed as color-coded change. Also, the GC-IPL thickness was displayed as superior or inferior and in total values in micrometers. We compared the numbers obtained to patients’ preoperative values. A significantly lower GC-IPL thickness was detected 6 months after surgery in the three regions that was more evident superiorly. Previous studies supported the reduction in the GC-IPL after PPV with ILM performed for either MH or epiretinal membrane surgeries.11,43 However, there are no previous reports of GCL changes using the enface OCT technique.

There are two possible explanations for the RNFL and GC-IPL defects. The first is cytotoxicity of indocyanine green dye (ICG) used during ILM peeling. There are many studies demonstrating the cytotoxic effect of ICG on ganglion cells.44–47 The second possibility is mechanical manipulation of the ILM, which damages the RNFL and GC-IPL. The presence of neuronal and ganglion cells on surgically excised ILM by immunohistochemistry supports this idea.48 In our study, ICG dye was not used, and BBD was applied to stain the ILM. Nevertheless, many studies supported the safety of this dye.49,50 Because the GC-IPL defects were well circumscribed and not diffuse, the hypothesis for the toxic effect of the dye on the GC-IPL could be eliminated, leaving only the mechanical manipulation as a possible explanation.

In our study, the postoperative BCVA improved significantly (P ≤ 0.001) despite the changes seen in the inner retinal layers with en face OCT (CMDS and GC-IPL defects) and significant GC-IPL thinning detected with the 3D volumetric OCT scanning. Because our study included only cases with successful anatomical MH closure, this suggests that restoration of retinal continuity plays a very important role in visual improvement. On the other hand, a previous study found a greater decrease in GC-IPL thickness was correlated with a worse postoperative BCVA after ILM peeling for epiretinal membranes with intact photoreceptor layers.43 Therefore, we can assume that even with successful anatomical MH closure, inner retinal defects may play a role in reduction or lack of improvement in the postoperative BCVA. We did not evaluate any functional parameter, such as correlation between BCVA and inner retinal defects, visual field, or retinal sensibility, and this might be considered a limitation of our study; however, our purpose was to report en face OCT inner retinal changes after ILM peeling for IFTMH and to correlate these findings with macular GC-IPL analysis. Additional research is needed to determine whether a relationship might exist between these functional parameters and the inner retinal changes after ILM peeling.

CONCLUSIONS

With ILM peeling, en face OCT scanning showed two forms of inner retinal layers changes. The first form is CMDS with intact GC-IPL. The second form is a combination of CMDS at the level of the
ILM with localized defects in the underlying GC-IPL. Ganglion cell-inner plexiform layer defects can be visualized on en face OCT display and correlate well with areas of thinning on the GC-IPL thickness maps derived from 3D volumetric OCT scanning.

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References

FIGURE 3. (A, B) Preoperative and postoperative en face OCT scanning at the level of the retinal nerve fiber layer, showing focal dehiscence corresponding to each dark spot in this layer postoperatively. Areas of focal dehiscence are very evident superio-temporally, but they can be seen all over the rest of the macula as well. (C, D) Preoperative and postoperative en face OCT scanning at the level of ganglion cell inner plexiform layer showing a visible defect superio-temporally. The defect appears darker than the surrounding area. (E) Print-out of the 3D volumetric OCT scanning showing marked thinning in the ganglion cell inner plexiform layer (GCL++) that correlates with defects seen with en face OCT scanning.