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ORIGINAL PAPER

Modified technique of internal limiting membrane staining in idiopathic macular hole surgery

Samir Mohamed El-Baha · Mahmoud Alaa Abouhussein

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Abstract
Purpose To describe a new method of ILM staining with TB under perfluorocarbon in cases of full thickness idiopathic macular hole using the inverted ILM flap technique.
Methods This study was a prospective interventional case series that included 42 eyes of 42 patients who had full thickness idiopathic macular hole with a minimum diameter more than 400 µm. Patients consecutively underwent vitrectomy with inverted ILM flap technique using the modified ILM staining method.
Results Anatomic success was achieved in 40 patients (95.2%). The other two patients had flat-open macular holes with bare RPE (foveal defect of neurosensory retina). Among the 40 eyes with closed holes, 25 eyes were of the U-type closure (normal foveal contour) and 15 eyes were of the V-type closure (steep foveal contour). These 40 eyes remained closed during the 6 months follow-up period.
Conclusion The modified technique of ILM staining using TB under PFCL is safe and effective in cases of idiopathic macular hole combined with the inverted ILM flap technique.

Keyword Internal limiting membrane · Staining · Macular hole · Inverted ILM

Introduction

Since its introduction in 1997, internal limiting membrane (ILM) peeling during vitrectomy for idiopathic macular hole was shown to increase the success rate of macular hole closure compared to surgery without ILM peeling [1–3].

Since the ILM is thin and difficult to visualize, surgeons found difficulties during ILM peeling: starting ILM peeling, completing the peeling if the edge is lost and assessing the extent of the peeled area [4].

ILM staining was used to easily identify the ILM and to avoid ILM peeling related complications. Different dyes were tried for this purpose such as indocyanine green (ICG), trypan blue (TB), or brilliant blue (BB).

ICG was the most used dye for ILM staining because it has high affinity for ILM and improves its visualization during surgery. Recently, there were studies reporting harmful effects of ICG on the retina. Trypan blue dye, which is widely used in anterior

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S. M. El-Baha · M. A. Abouhussein (✉)
Ophthalmology Department, Alexandria University, 8 Hassan Allam Street, Kasr Elmoltazem, Smouha, Alexandria, Egypt
e-mail: nad03alaa@yahoo.com

segment surgeries, is safer than ICG, but it does not stain the ILM as good as ICG [5].

Two published studies evaluated the anatomic and visual outcomes after vitrectomy and ILM peeling for idiopathic macular hole repair in patients with stage II–IV idiopathic macular holes using ICG or TB. Results showed similar macular hole closure rates but better visual results in TB cases [6, 7].

The decreased visual outcome with ICG was proposed to be due to possible damage of the retinal pigment epithelium (RPE) layer [8, 9].

Several methods have been tried to enhance the ILM staining effect of TB such as injection of TB under air or mixing TB with glucose 10% [5].

The inverted ILM flap technique was introduced in 2010 by Michalewska et al. [10]. This technique was shown to reduce the flat-open appearance after surgery for large idiopathic macular hole. It also showed improved foveal anatomy as shown by optical coherence tomography (OCT).

We describe a new method of ILM staining with TB under perfluorocarbon in cases of full thickness idiopathic macular hole using the inverted ILM flap technique.

Patients and methods

This study was a prospective interventional case series that included 42 eyes of 42 patients (32 women and 10 men) who had full thickness idiopathic macular hole with a minimum diameter more than 400 μm. Patients consecutively underwent vitrectomy with inverted ILM flap technique using the modified ILM staining method. All cases were operated upon by a single surgeon (S.M.E.) using the same technique.

The study was approved by the Alexandria university ethics committee. All patients gave consent to participate in the study. The research followed the tenets of the Declaration of Helsinki.

Exclusion criteria were secondary macular holes, conditions affecting the retina as diabetic retinopathy and age-related macular degeneration, history of intraocular operations other than cataract surgery.

Preoperative evaluation included:

History of symptoms duration, complete ophthalmological examination including best-corrected visual acuity using Snellen chart and fundus biomicroscopy using 78-D non-contact lens and OCT using commercially available spectral domain OCT (SD-OCT): Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA).

The best-corrected visual acuity (BCVA) was converted to a logarithm of minimal angle of resolution Log MAR for statistical analysis.

Surgical technique

All cases were operated upon by the Constellation vision system (Alcon-USA) 23-gauge system.

Complete 23 gauge transconjuctival vitrectomy with removal of posterior hyaloid face was done.

Perfluoro-*n*-octane (C8F18; adato-octa, Adatomed, München, Germany) was injected slowly as a single large bubble over the posterior pole with a 23-gauge double-barrel cannula (Midone). Then, 0.1 ml of commercially available trypan blue 0.15% (MembraneBlue, DORC) was injected over the macula at the retina–perfluoro-*n*-octane interface. TB was left for 1 min.

The internal limiting membrane was stained blue and became clearly visible under the perfluoro-*n*-octane. The dye also stained the retinal pigment epithelium within the hole without passing to the subretinal space. The inverted ILM flap technique was performed according to the method described by Michalewska et al. [10]. The internal limiting membrane was peeled in all patients using disposable microforceps ILM 23 gauge/0.6 (1286, DORC, Geervliet, The Netherlands) (see Video, Supplemental Digital Content 1, which demonstrates our technique). Figures 1 and 2 show intraoperative photographs of surgical steps.

Screening of the peripheral retina for possible peripheral breaks by peripheral indentation was done.

The perfluoro-*n*-octane–air exchange was then performed, and intraocular volume was replaced with 20% mixture of sulfur hexafluoride (SF6).

Patients were asked to keep a facedown position postoperatively (16 h per day) for 5 days.

The follow-up period was 6 months after surgery. Patients were evaluated on the next day postoperatively, after 1, 3 and 6 months.

Best-corrected visual acuity was recorded and OCT was performed at the 1, 3 and 6 months visits.

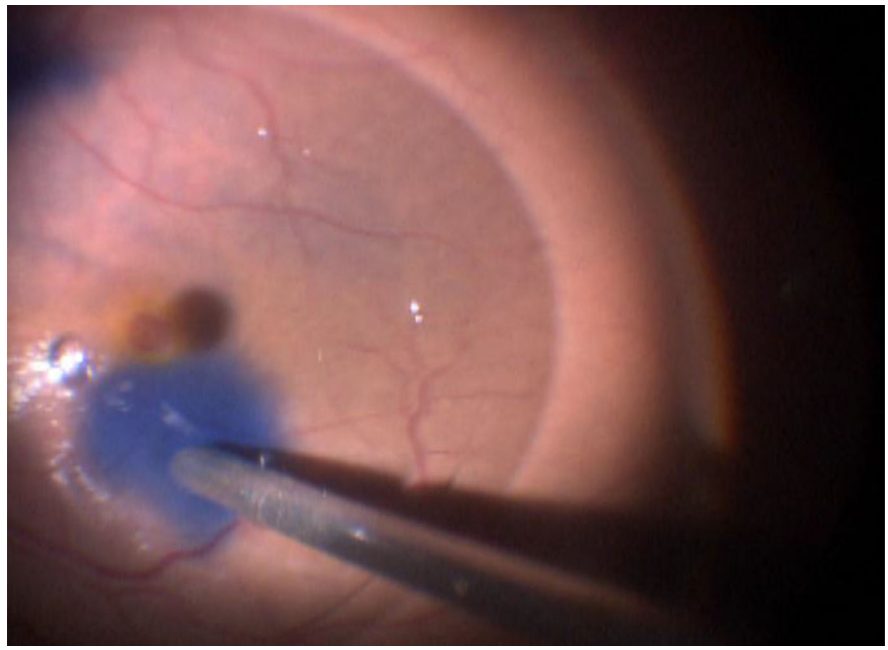

Fig. 1 Intraoperative modified ILM staining technique

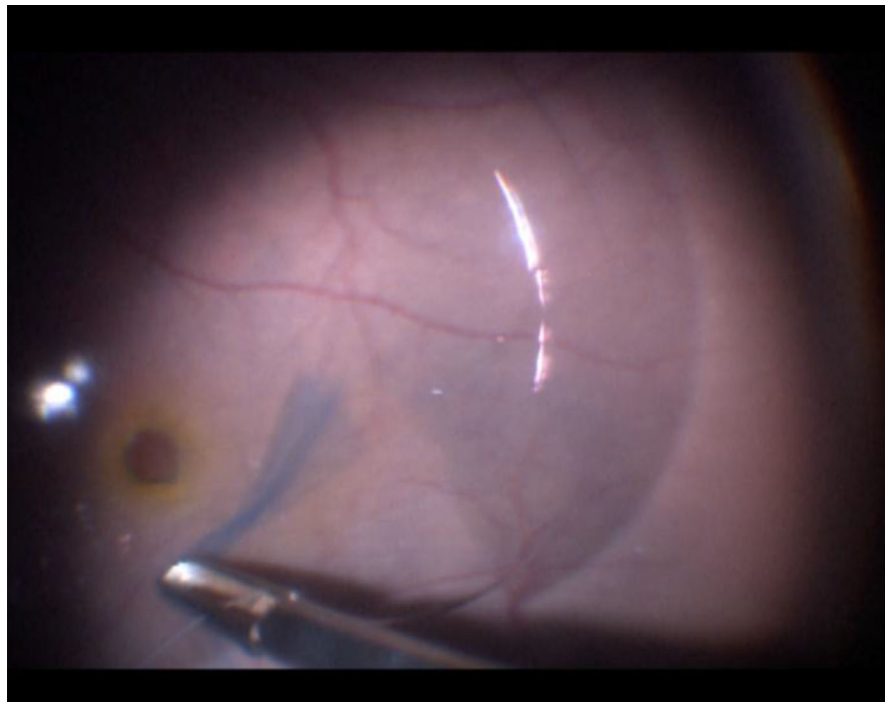

Fig. 2 ILM peeling

Anatomic success was defined as the closure of the macular hole at the 1-month evaluation visit as indicated by clinical examination and OCT.

Statistical analysis was done using Statistical Package for Social Sciences (SSP/Version 20, LEAD Technology, USA) software. Probability value of < 0.05 was considered to be significant.

Results

The study was carried on 42 eyes of 42 patients (32 women and 10 men) with full thickness idiopathic macular hole of a minimum diameter more than 400 μm. The mean age was 65.3 ± 5.68 years (range from 55 to 79 years). Twenty-five patients were phakic, and 17 patients were pseudophakic.

The mean duration of symptoms was 6.43 ± 8.23 weeks (range from 1 to 52 weeks). Twenty-five patients (59.5%) had less than 4 weeks duration of the visual complaints, while 17 patients (40.5%) had more than 4 weeks duration of symptoms.

Preoperative evaluation showed that the mean minimal macular hole diameter was 646 μm (ranging from 480 to 930 μm).

The surgery was done in all cases successfully with no intraoperative complications. Adequate staining of the ILM by this method was done in all cases, the ILM was easily visible under the perfluoro-*n*-octane and there was no need to restain the ILM in any case. Passage of trypan blue into the subretinal space through the macular hole did not occur.

Anatomic success (closure of the macular hole at the 1-month evaluation visit as indicated by clinical examination and OCT) was achieved in 40 patients (95.2%). The other two patients had flat-open macular holes with bare RPE (foveal defect of neurosensory retina). Regarding the OCT findings among the 40 eyes with closed holes, 25 eyes were of the U-type closure (normal foveal contour) and 15 eyes were of the V-type closure (steep foveal contour). These 40 eyes remained closed during the 6 months follow-up period.

No postoperative complications (rhegmatogenous retinal detachment, epiretinal membrane, vitreous hemorrhage, endophthalmitis or increased intraocular pressure) were recorded. Cataract progression was recorded in two cases at the 3 months follow-up visit and cataract surgery was done.

At the 6-month visit, OCT findings included: 10 patients with focal defects in IS/OS junction layer, 4 patients with nerve fiber layer defects and 2 patients with foveal detachment. RPE abnormalities were not noted either clinically or on postoperative OCT scans in any patient.

As regards the visual outcome of the patients, the mean preoperative LogMAR visual acuity was 0.94 ± 0.30 SD (range from 0.31 to 1.49). At the 6 months follow-up visit, the mean LogMAR visual acuity was 0.66 ± 0.37 SD (range from 0.23 to 1.46). These results were statistically significant, with a P value of 0.007. At the end of 6 months follow-up, visual improvement occurred in 40 patients and visual stabilization occurred in two patients (these two patients had failed anatomic closure and refused further interventions).

Discussion

The term chromovitrectomy was used since the year 2000 to describe the use of vital dyes in staining the retinal and preretinal tissues to facilitate intraoperative steps during vitrectomy [11].

ICG was the first dye that was introduced to stain the very thin ILM [12].

However, there were multiple reports from in vivo and in vitro studies that pointed to the possible toxic effects of ICG on the retinal tissues [2]. Central visual field changes, RPE damage and optic atrophy were documented after chromovitrectomy using ICG [2, 8, 11].

Trypan blue (TB) was suggested as a safer alternative for chromovitrectomy to stain epiretinal membrane (ERM) and ILM [13].

TB use began initially in cataract surgery to stain the anterior lens capsule (0.06% trypan blue in VisionBlue, DORC, Zuidland, The Netherlands) [14]. At a higher concentration, TB can be used in staining preretinal structures (MembraneBlue DORC 0.15%) [15].

TB has some advantages over ICG: TB appears to be safer and it was shown to be non-toxic to cultured RPE cells and glial cells. Another study showed that it is non-toxic to rabbit retina when it is promptly removed [13, 16–19]. TB is also less expensive than ICG and easier to remove due to its high water solubility [20].

TB has high binding affinity for tissues with high cellular proliferation. That is why it produces good staining effect of ERM which has cellular components formed of astrocytes and myofibroblasts in addition to collagen framework [5].

On the other hand, because the ILM is an acellular membrane, it is not brightly stained with TB compared to ICG. So when used to stain the ILM, TB has to stay

a longer time in contact with ILM compared to ICG [11, 21].

To overcome this problem, two approaches were tried. The first one is to inject TB under air after fluid air exchange to avoid the diluting effect of fluid filled vitreous. When TB is used without fluid air exchange it does not stain ILM sufficiently. The second approach to mix the TB with 10% glucose solution forming a heavy mixture which produces better staining effect [22, 23].

However, higher glucose concentrations should be avoided since glucose 50% has a highly toxic osmolarity of 1150 mOsm [24].

In this study, as a trial to enhance TB staining effect, we used a technique of ILM staining with TB under perfluorocarbon in vitrectomy for idiopathic macular hole using the inverted ILM flap technique. Our results showed that with this technique, TB produces a good staining effect of the ILM facilitating the ILM peeling step.

The initial trial of using TB to stain the ILM in cases of macular hole surgery dates back to 2002. In this retrospective study, 0.5 to 1 ml of TB 0.06% was injected under fluid infusion over the posterior pole under direct visualization, staining the ILM. The results showed good anatomic and visual benefits with no clinically evident retinal pigment epithelial changes [25].

The concentrations of TB used in vitrectomy ranges from 0.06 to 0.2%. The injected volume range from 0.1 to 1 ml. The TB exposure duration range from 1 to 2 min [4]. In this study, we used the 0.15% concentration and exposure time of 1 min.

Oberstein et al. [26] mixed TB with 10% glucose to produce what they termed heavy TB. Heavy TB was prepared by mixing equal volumes of glucose 10% with commercially available TB 0.15% (Membraneblue, DORC). The mixture had a glucose concentration of 5% and a TB concentration of 0.075%. The osmolality of this solution was 320 mOsm with neutral pH. They used this mixture to stain ERM staining without fluid–air exchange. Their study included 29 eyes. All patients showed visual acuity gain with no apparent retinal toxicity, but in 25% of eyes reapplication of heavy TB was needed to achieve ERM staining.

In 2010, heavy TB was used for ILM staining in cases of full thickness macular holes. Results showed the need for reapplication of dye in 75% of the cases [27]. In our study, using the modified ILM staining technique, we did not need to restain in any of our cases.

Perfluocarbon liquid (PFCL) is a high-density, low-viscosity substance which is used as a temporary endotamponade in vitreoretinal surgery [28].

In chromovitrectomy, PFCL was used to tamponade the macular hole in order to prevent ICG contact with the RPE and to avoid ICG entrance to the subretinal space [29]. Two studies used PFCL with ICG staining of ILM for this purpose. They reported good anatomic and visual outcomes with no signs of RPE defects [30, 31].

In our study, we used the PFCL to be able to enhance the TB staining effect on the ILM without fluid air exchange and to facilitate the peeling procedure. The posterior countertraction of the perfluoro-*n*-octane facilitates the peeling of the stained ILM. It also prevents the diffusion of the dye into the subretinal space or in the vitreous cavity. The traction over the macular is minimal avoiding enlargement of the macular hole from attempted ILM peeling. The aim was not to prevent TB contact with the RPE within the macular hole. Actually, the RPE within the hole was mildly stained with no apparent toxic effects.

In our study, we found that the use of TB with this technique was safe, with no clinical or OCT signs of retinal toxicity. The good anatomic and visual results are encouraging.

This technique has some negative points. The increased cost of using PFCL and the need for meticulous PFCL removal to prevent toxic effects of retained PFCL.

In conclusion, we find this modified technique of ILM staining using TB under PFCL to be safe and effective in cases of idiopathic macular hole combined with the inverted ILM flap technique.

Compliance with ethical standards

Conflict of interest Both authors declare that they have no conflict of interest.

Research involving human participants Alexandria University Ethical Committee approval was obtained, research was adherent to Declaration of Helsinki.

Informed consent Informed consent was obtained from all patients.

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