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Internal limiting membrane peeling in macular surgery-morphological and functional outcome

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LIST OF ABBREVIATIONS

- BBG : Brilliant Blue G.
- BRVO : Branch Retinal Vein Occlusion.
- BSS : Balanced Saline Solution.
- CRVO : Central Retinal Vein Occlusion.
- ERG : Electroretinogram.
- ERMs : Epiretinal Membranes.
- ICG : Indocyanine Green.
- ILM : Internal Limiting Membrane.
- IMH : Idiopathic Macular Hole.
- MP1 : Microperimetry.
- MVR : Microvitreoretinal Knife.
- OCT : Optical Coherence Tomography.
- PVD : Posterior Vitreous Detachment.
- RPE : Retinal Pigment Epithelium.
- VMTS : Vitreomacular Traction Syndrome.

1. INTRODUCTION

1.1 The eye ball:

The human eye is an organ which reacts to light for several purposes. As a conscious sense organ, the eye allows vision. Rod and cone cells in the retina allow conscious light perception and vision including color differentiation and the perception of depth. The human eye can distinguish about 16 million colours. [1]

1.1.1 The Retina:

Anatomically the retina is a delicate thin transparent structure covering the inner surface of the posterior part of the eye ball. It is loosely attached to the pigment epithelium being closely bound down at the optic disc and at the orra serrata.[2]

1.1.2 Subdivisions of the retina:

The retina can be subdivided into 2 regions [3]

1-Central area measuring 5-6 mm in diameter, concerned with diurnal vision including appreciation of colors.

2-Peripheral large area surrounding it comprising the rest of the retina, concerned with summation of weak stimuli as occurs in dim illumination and the perception of movements.

1.1.3 Macula Luta:

Anatomically, the macula is defined as an area of the posterior retina containing xanthophylls. Clinically, this area is five mm in diameter and its center is located four mm temporal and 0.8mm inferior to the center of optic nerve head. It occupies most of the area between the vascular arcades. [2, 3]

1.1.3.1 Subdivisions of the Macula:

1.1.3.1.1 Fovea Centralis:

This is a depression in the inner retinal surface at the center of the macula. Its diameter is 1.5 mm as the diameter of an average optic disc head. Clinically, it can be recognized with an oval light reflex arising from an increased thickness of the retina and the internal limiting membrane. [2, 3]

1.1.3.1.2 Foveola:

It is the central part of the fovea. It measures 0.35 mm in diameter and lies within the capillary free zone. It is the thinnest part of the retina and devoid of ganglion cells. Its entire thickness consists of only cones and their nuclei. It delivers the most acute visual acuity. The umbo is a tiny depression of the center of the foveola. [2, 3]

1.1.3.1.3 Histology of retina. [2, 3]

The retina is formed of ten layers, which are from out to inwards:

1-Retinal pigment epithelium (RPE).

2-Layer of rods and cones.

3-External limiting membrane.

4-Outer nuclear layer.

5-Outer plexiform (fiber) layer.

6-Inner nuclear layer.

7-Inner plexiform (fiber) layer.

8-Ganglion cell layer.

9-Nerve fiber layer.

10-Internal limiting membrane (ILM).

1.2 Anatomy of the internal limiting membrane:

The internal limiting membrane (ILM) is the innermost layer of the retina. It forms a boundary between the vitreous and the retina and is a part of the vitreoretinal interface. The ILM also acts as a structural support for the Müller cells of the retina. [4]

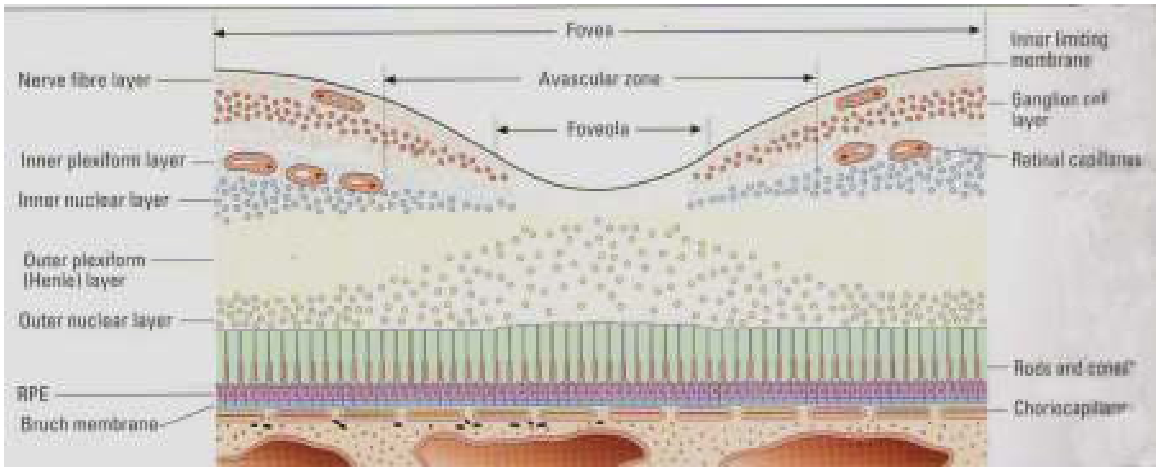

Fig 1. Cross section of normal fovea. (Kanaski J 2003). [5]

1.2.1 Thickness and continuity:

The thickness of the ILM varies from 100-200 nanometers in the anterior retina. Posteriorly, the ILM attains a thickness of 0.05-2.0 micrometer. It continues uninterrupted at the fovea. It is absent at the edge of the optic disc. [6]

At the periphery of the retina, the membrane is continuous with the basal lamina of the ciliary epithelium. The basal lamina shows breaks at the pars plana and at the ciliary processes where vitreous fibrils are in direct contact with the cell membranes of the epithelial cells. These breaks in the basal lamina increase with age. The ILM becomes thicker and interrupted at the ora serrata with age. [6]

1.2.2 Structure:

Both the retina and the vitreous contribute to the formation of the ILM and vitreoretinal interface. [5]

The vitreous portion of the ILM appears smooth in flat sections of the retina, except at the retinal periphery where it may be irregular. [7]

1.3 Anatomy of the vitreous:

The vitreous body fills the eyeball behind the lens. It thus occupies about four-fifth of the eyeball and lies between the lens and the retina. [7, 8]

The vitreous body is firmly attached to the retina in some regions: vitreous base, margin of the optic disc, margin of the macula and overlying retinal vessels. Elsewhere, the attachment between the retina and the vitreous is loose. [9, 10]

The function of the vitreous is transmission of light and contributes slightly to the dioptric power of the eye. It supports the posterior surface of the lens and possibly assists in holding the neural part of the retina against the pigmented part. The vitreous probably plays an important role in retinal metabolism by serving as a repository for chemical substances and influencing the movements of solutes and solvents. Although, the absence of vitreous (after a surgical procedure, called vitrectomy) does not affect the retinal function. [2, 10]

1.4 Macular holes

Full thickness macular holes are defined as defects involving all layers of the retina, from the internal limiting membrane through the outer segment of the retinal photoreceptors in the foveal area. Lamellar macular holes involve only a portion of retinal layers. [11]

The most common cause of full thickness macular hole is the idiopathic macular hole. Other causes include traumatic macular hole, pathological myopia, solar retinopathy, vitreomacular traction syndrome, traction from epiretinal membranes and degenerative conditions of the retina. [12, 13, 14]

1.4.1 Idiopathic macular hole (IMH):

Gass in 1988 proposed that tangential traction caused by shrinkage of premacular vitreous cortex is responsible for idiopathic macular hole formation. The process typically begins in eyes with liquefied pockets of premacular vitreous and no posterior vitreous detachment (PVD). This suggests that (IMH) begins as foveal cystic changes with unroofing of the hole. Scanning electron microscopic studies have shown that even after apparent spontaneous PVD, there are remnants of posterior vitreous membrane in the foveal area. These glial cells can create tangential traction leading to formation of idiopathic macular hole.[13]

Clinical stages of macular hole formation:

Gass described a series of chronological stages of fundus changes in the majority of cases of idiopathic macular holes.[13] These clinical stages originally were described in a retrospective series. [15, 16]

Stage (1a): A yellow spot 100-200 μ in diameter is centered on the Foveola. This stage is not pathognomonic of idiopathic macular hole formation. It is caused by localized shrinkage of perifoveal vitreous cortex and is also seen in cases of:

*Central serous chorioretinopathy

*Aphakic cystoid macular edema

*Solar retinopathy

Associated loss or reduction in the fovea depression occurs. This lesion may be the same as that described by others as cyst. [17, 18]

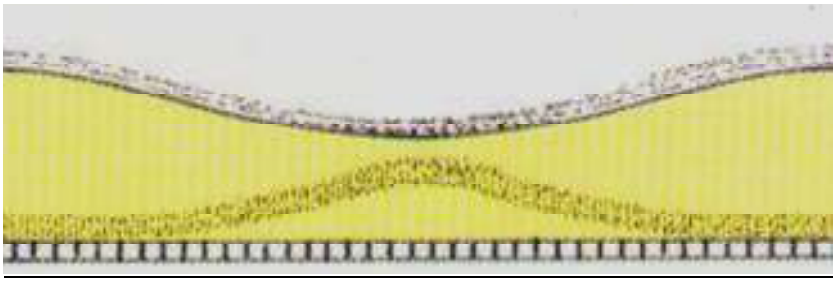

Fig 2. Stage 1 A impending macular hole. (Kanaski J 2003). [5]

Stage (1b): A yellow ring 200-300 μ in diameter and also is centered on the foveola, this finding appears to be specific to macular hole formation, patients of this stage are often asymptomatic, with a diminution of visual acuity with metamorphopsia caused by further vitreous contraction leading to tiny breaks in the photoreceptors in the center of the foveola but with intact ILM.

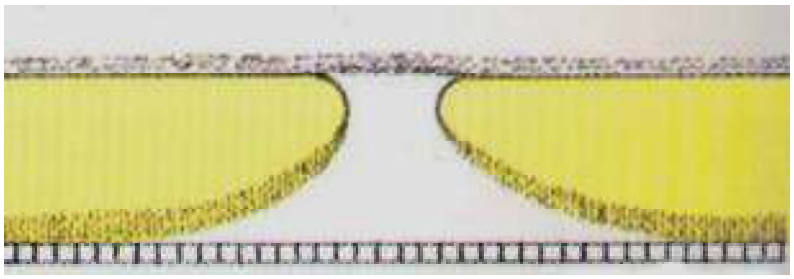

Fig 3. Stage 1 B occult macular hole (Kanaski J 2003). [5]

Fate of stage 1 macular hole: Stage 1 lesions are transient, they resolve within few weeks owing to spontaneous vitreofoveal separation. Resolution may be accompanied by improvement of vision and metamorphopsia. A semitranslucent premacular opacity may be visible biomicroscopically representing contracted vitreous cortex

Stage (2): Caused by condensation of perifoveal cortical vitreous with separation from the retinal surface to form pseudo-operculum. It is the first evidence of full thickness retinal defect. This change usually occurs at the inner edge of the yellow ring.

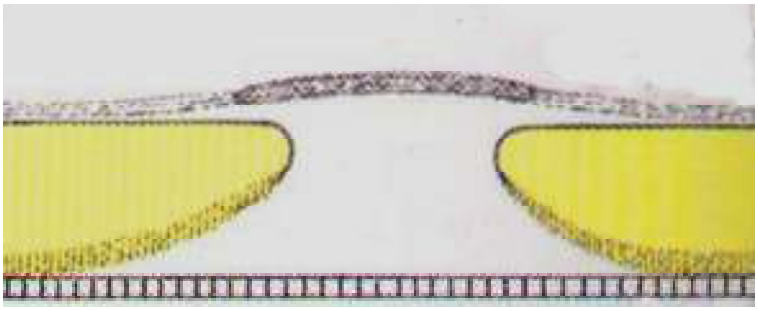

Fig 4. Stage 2 Macular hole (Kanaski J 2003). [5]

Fate of stage 2 macular hole: The macular hole enlarges over a period of days to weeks to stage 3 or 4. Eyes with eccentric holes and those with pericentral hyperfluorescence are more likely to progress than those with centric holes.

Stage (3): It is defined as a fully developed macular hole of 400 um or more in diameter with vitreofoveal separation. In 75% of cases an opacity can be detected on the detached posterior vitreous face and anterior to the macular hole. This opacity was initially thought to be an operculum.

The underlying retinal hole continues to enlarge, presumably because of centrifugal retraction of retinal tissue. The underlying retinal pigment epithelium (RPE) within the area of retinal hole formation shows thinning and depigmentation. This contributes to hyperfluorescence seen on the fluorescein angiography.

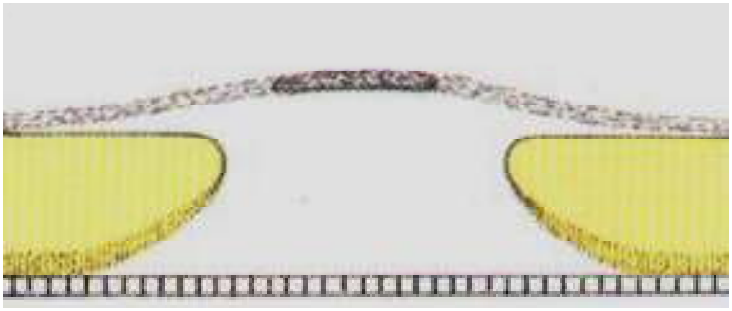

Fig 5. Stage 3 macular hole (Kanaski J 2003). [5]

Stage (4): It is defined as full thickness macular hole with complete posterior vitreous detachment with the presence of Weiss ring infront of the optic nerve head.

Fate of stage 3 and 4 macular hole: Visual acuity stabilizes at about 0.1. Spontaneous closure is rare but may be caused by growth of an epiretinal membrane. [19]

Fig 6. Stage 4 macular hole (Kanaski J 2003). [5]

1.4.2 Traumatic macular hole:

Initially trauma was believed to be the major cause of macular hole formation. [20] The exact mechanism of formation of macular hole after blunt trauma is uncertain. [21]Yanagiya et al., theorized that the forces of impact transmitted to the macula caused foveal rupture and they supported their theory by the elliptical or irregular appearance of the edges of the macular hole. [22] Others theorized that countercoup forces led to sudden vitreal separation and traction on the macula with hole formation. [23] Another theory suggested that trauma causes rupture of a previously developed macular cyst with subsequent hole formation. [22, 23, 24]

Johnson et al., [21] proposed the following mechanism: blunt trauma leads to indentation of the cornea followed by outward expansion of the globe at the equator and flattening of the posterior pole followed by posterior displacement of the posterior pole.

Sudden flattening, then posterior displacement of the posterior pole exerts sudden traction on the anatomically thin fovea resulting in immediate hole formation. This traction was described as tangential and the vitreous has a role in its development. [24]

1.4.3 Myopic foveoschisis and myopic macular hole:

Myopic foveoschisis is the splitting of retinal layers in the macula and can result in metamorphopsia, blurring of vision and predisposes to myopic macular holes. Myopic foveoschisis and macular holes causing retinal detachments are more common with high myopia. [25, 26, 27] Since the fovea and to a lesser extent the macula are dependent upon the blood supply of these deeper layers, a relative ischemia can exist in the sensory retina. Furthermore, staphyloma formation with accentuated stretching of the posterior pole causes thinning of the retina with distinct flattening of the macular area probably because of thinning of the ganglion cells layer. Cystic degeneration, schisis formation then atrophy of the macula may ensue with the possibility of macular hole formation. [28]

1.5 Macular pucker

Macular pucker has also been called cellophane maculopathy, epiretinal membranes and premacular fibrosis. In adults, these lesions have been described as an idiopathic occurrence as well as secondary to intraocular inflammation, macular holes, trauma, retinal detachment, retinal vascular disease and interestingly diabetes without evidence of retinopathy. [29]

1.5.1 Histopathology:

In 1981, Kampik using transmission electron microscope defined five basic cell types present in epiretinal membranes: retinal pigment epithelial cells, macrophages, fibrocytes, fibrous astrocytes, and myofibroblast-like cells. The RPE cells were only noted in association with retinal detachment. [30]

A posterior vitreous detachment, commonly associated with idiopathic epiretinal membranes, may precipitate the formation of these membranes by causing defects in the ILM. [31] It is presumed that glial cells, which have direct access to the internal surface of the retina through breaks in the ILM, may proliferate or undergo transformation into other cell types, forming epiretinal membranes. [31] A new theory discusses the role of a pathologic posterior vitreous detachment in which parts of the vitreous cortex remains attached to the retinal surface. Cells within this collagenous layer may proliferate and finally contribute to tractional forces at the retinal surface. [32] Epiretinal membranes may contain myofibrocytes which may exhibit their contractile properties in the clinical feature of prominent retinal striae especially in younger patients. [33]

Increased numbers of fibrous cells and collagen may correlate with thicker and whiter membranes, which are more found in young patients. [34]

1.5.2 Idiopathic epimacular membranes:

The incidence of idiopathic membranes increases with age. Idiopathic membranes are very rare in children and adolescents. [35] Metamorphopsia, distortion of amsler grid pattern and reduction of the visual acuity occur once the fovea is involved. As retinal traction develops, the small vessels within the temporal vascular arcades may become tortuous. The large retinal vessels are usually normal early in the disease process, but with progression they may become darker, dilated and tortuous. The disease process may advances rapidly over few months but more commonly progresses slowly over long periods of time. [36]

1.5.3 Secondary epimacular membranes:

Secondary epimacular membranes may occur in patients who have undergone previous retinal surgery. Several preoperative and intraoperative factors have been associated with increased incidence of epimacular membrane formation after retinal reattachment surgery. These factors include increased patient age, vitreous hemorrhage and drainage of subretinal fluid. [37]

1.5.4 Clinical stages of epiretinal membranes:

In 1987 Gass offered the following classification: [36]

Grade 0: Translucent membranes unassociated with retinal distortion (cellophane maculopathy).

Grade 1: Membranes causing irregular wrinkling of the inner retina (Crinkled cellophane maculopathy).

Grade 2: Opaque membranes obscuring the underlying vessels with prominent retinal distortion.

1.6 Vitreomacular traction syndrome

The hallmark of a vitreomacular traction syndrome is a persistent attachment of the vitreous to the macula in eyes with an incomplete posterior vitreous detachment. The most common morphological configuration is a vitreous separation peripheral to a zone where the cortical vitreous remains attached to the retina at the macula and the optic nerve head. Traction on the macula causes decreased vision, metamorphopsia, photophobia and micropsia.[38]

There are remarkable variations concerning the vitreoretinal morphology in eyes with vitreomacular traction syndrome. This syndrome comprises a broad spectrum of frequently unrecognized clinical findings, ranging from peripheral vitreous separation with residual foveal attachment to multiple areas of tractional retinal detachment caused by persistent, focal posterior and peripheral vitreous attachment. [39, 40, 41]

Based on the relationship of the posterior vitreous cortex to the retina in cases of epimacular membranes and according to its clinical effect, Akiba in 1991 classified this relationship into four groups. [42]

Group 1: No posterior vitreous detachment.

Group 2: Partial posterior vitreous detachment but no vitreoretinal adhesion or traction to the area of the preretinal macular fibrosis.

Group 3: Partial posterior vitreous detachment with vitreous traction to the area of the preretinal macular fibrosis.

Group 4: Complete posterior vitreous detachment.

1.6.1 Histopathology:

Vitreomacular traction membranes revealed fibrocellular membranes composed of fibrous astrocytes, fibrocytes, myofibroblasts, collagen and fragments of the internal limiting membrane. [43]

1.7 Indications for surgical ILM peeling

1.7.1 ILM peeling in macular pucker and in vitreomacular traction:

Peeling of ILM has been indicated in cases of epimacular proliferation. Removal of epimacular proliferation/ILM may help for complete tractional release with high anatomical and functional success rate. [44, 45]

1.7.2 ILM peeling in macular hole:

In cases of idiopathic macular hole, ILM peeling was found to be effective not only in hole closure but also in visual recovery. ILM peeling was suggested to improve visual and anatomic success in all stages of macular holes. [46, 47]

The ILM peeling is intended to remove cellular proliferation on the ILM in IMH and to release tractional forces generated by contraction of these cells and almost remove the scaffold for the proliferation of glial cells. [48]

Another indication for ILM peeling is the myopic macular hole with retinal detachment. In such cases, removal of ILM can result in a high initial success rate of retinal reattachment. [49]

1.7.3 Other indications for ILM peeling:

Recent studies and reports have described the effectiveness of ILM removal in pseudo-hole cases in myopic eyes, [50] vein occlusive diseases such as central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO). [51] It has been recommended that ILM peeling be performed after silicone oil removal to prevent late postoperative complications such as secondary macular pucker. [52]

1.8 Techniques of ILM peeling

Several techniques have been described to achieve this surgical step of ILM peeling during vitreous surgery. Neither of these techniques have proved to be exclusively the best nor the easiest nor the safest among others. The technique is still left to the surgeon's preference and skills.

1.8.1 The first technique:

It was described by Rice in 1999. [53] After complete vitrectomy and removal of the posterior hyaloid, ERMs are excised as much as possible. The ILM is then punctured with a sharp tipped Eagle dissecting needle or a bent tipped MVR (70-90°). The needle is tilted slightly downwards 15-20° to engage the ILM. Tano's diamond dusted membrane scraper is then introduced with the tip directed tangentially into the opening of the ILM and beneath it. The scraper is then advanced slowly in an effort to engage the ILM and not the nerve fiber layer. If a translucent or even subtle white membrane is seen over the instrument, the latter is in the nerve fiber layer and hence too deep. The instrument is drawn back and readvanced in the proper plane. If this is not possible, another site is picked and punctured with the needle or microvitreoretinal knife (MVR).

Sometimes the ILM may be seen over the elevator as clear cellophane like sheet and sometimes nothing is seen, in this case advancing or lifting the instrument will cause movement of the underlying retina or retinal striae.

Once the ILM elevator is in proper plane it is advanced slowly in a straight line towards a point approximately 3 mm to the left of the macular hole in the horizontal meridian, and at that point the instrument is directed more vertically.

Once a sheet of the ILM is elevated the instrument is withdrawn and the ILM is grasped by an endgripping forceps that prevents shredding of the grasped ILM and then it is peeled off like the anterior lens capsule in capsulorrhexis. Sometimes the ILM is firmly adherent, that is why care must be taken to keep the traction force horizontal (tangential to retina). Every effort should be made to peel off the ILM in one piece and not to shred it.

1.8.2 The second technique:

This technique differs from the first one that it does not involve the use of the ILM elevator. The surgeon starts by making a small opening and a flap tear in the ILM by a bent tipped MVR. Then, the endgripping forceps is introduced to grasp the flap and to proceed with peeling directly without creating a plane between the ILM and nerve fibre layer. It can be done in a continuous circular motion. Some surgeons do not remove the ERMs and leave them to be removed with ILM in a single step to minimize manipulation during surgery. [46]

1.8.3 The Third technique:

This describes a fluidic dissection of the ILM. It is considered by some to be a less traumatic way of removing the ILM in all forms of traction maculopathy. It consists of a viscoelastic syringe and tubing that connect to a viscous injector. There is a 20 gauge hand piece that supports the smallest cannulated needle. The needle is rectangular in shape with a short bevel sharpened on the bottom surface. This shape creates a tiny dissecting surface that is parallel to the retina and allows easy cannulation of the sub-ILM space. Using the injector at a low pressure, a small elevation of the ILM is created. Once the sub-ILM space is cannulated, the needle is slowly advanced while the pressure is increased. This allows for smooth and rapid dissection of the ILM with the overlying proliferation. After the ILM is elevated, it can be removed with ILM forceps without touching the retina. [54]

The technique used in this series of cases according to 2nd techniques is less traumatic.

1.8.4 Staining techniques of the ILM:

The peeling of the internal limiting membrane and epiretinal membrane is a challenging maneuver in vitreoretinal surgery. Some of the technical difficulties usually encountered during ILM peel surgery are incising or grasping the ILM surface; finding the edge of the initial incision on the ILM creating a flap; and may be most importantly, identifying the areas where the ILM has already been peeled. Epiretinal membranes may be difficult to visualize if fibrosis or pigment deposition is absent, and as a result, the removal of the membrane may be incomplete. Inadvertent trauma to the retina may also occur, in particular during ILM peeling. [55]

1.8.4.1 Indocyanine Green Dye (ICG):

ICG is a hydrophilic dye used for angiography because of its properties as a fluorophore. Indocyanine green also acts as a chromophore staining the ILM green because of its affinity to laminin and collagen type IV within the ILM. [56] It is the most potent and specific ILM stain used in macular hole surgery. [57] The most commonly reported adverse events after ICG –assisted ILM peeling are visual field defects. [58-66]

Several mechanisms related to ICG induced ocular toxicity include:

1- A direct dose dependent biochemical injury to RPE cells and ganglion cells.[67-68-69]

2- Osmolarity effect of the ICG–solution at the vireo-macular interface, indicating that hypotonic ICG solutions could be harmful all to the RPE. [70]

3- Phototoxic properties of the ICG inducing photo-oxidative cell damage caused by an overlap in absorption spectra between ICG peak (780-830 nm) and different types of endoillumination used in vitreoretinal surgery. [71-72]

1.8.4.2 Trypan Blue:

Trypan blue is a vital stain used in surgery for removal of epiretinal membranes and to stain the anterior lens capsule during cataract surgery. Trypan blue also stains cells with damaged cell membranes in a dose dependent matter. [73-74]

The faint bluish staining seen after trypan blue exposure is probably because of the affinity to cellular proliferations on the internal limiting membrane rather than to the acellular internal limiting membranes. [75]

Trypan blue staining is unlikely to have toxic side-effects when used in low and clinically relevant concentrations. [76]

1.8.4.3 Brilliant Blue G (BBG):

Brilliant blue is a blue biostain also known as acid blue 90 and coomassie brilliant blue G, that provides selective staining of the ILM. The dye is relatively new (approved in Europe in 2006). [77]

Enaida et al., 2006 examined the effect of brilliant blue G (BBG) stain on rats. After intravitreal BBG, no toxic effects of BBG, such as corneal edema, severe retinal edema or endophthalmities were observed by surgical microscopy over a period of 2 months. Normal structure of the retina was preserved in eyes injected with high doses of BBG (10mg/ml) with no infiltration of inflammatory cells observed. Regarding the electroretinogram (ERG), which was done to evaluate the retinal function, no remarkable reduction in ERG amplitudes, were observed. [78]

1.9 Evaluation of the function of the macular area

For many years, ophthalmologists have been using standard visual acuity testing to evaluate the function of the macular area. Recent advances in medical and surgical options of macular diseases have changed treatment modalities and therefore improved the prognosis of macular diseases. Therefore, more exact evaluation and documentation of macular function has become essential. [79]

1.9.2 Microperimetry (MP1):

The Micro Perimeter (MP1, Nidek instrument Inc., Padova, Italy) is not a scanning laser ophthalmoscope. Rather, the fundus image is observed using an infrared fundus camera with 45° field of view.

Microperimetry is a recent tool to evaluate the function of the macula in detail. The first Microperimetry was performed via a scanning laser ophthalmoscope. This device was not used for this study.

Perimetry using the MP1 is performed with a liquid crystal display controlled by a special software. The major advantage compared to the scanning laser ophthalmoscope is the automatic eye tracking, which allows real-time compensation for eye movements and therefore presentation of any stimulus exactly at the predefined retinal location. During stimulus presentation, the eye tracker monitors the eye position with the frequency of 25Hz i.e. each 40 ms. If the reference area has moved due to eye movement and can be found, the stimulus will be replaced accordingly. If the reference area can not be found, the stimulus will turned off. [79]

In the mean time, a color fundus photograph can be acquired by the MP1 following testing, and the result of the examination can be registered either automatically or manually with the infrared image.

The current MP1 software enables the examiner to choose between a symbolic, a numeric or an interpolated presentation of the resulting differential light thresholds.

The diameters of the stimuli can be altered between sizes comparable to conventional goldmann-perimeter size I and V. Differential light threshold is measured

with 4-2-1 staircase strategy in which stimulus intensity is increased or decreased (Fig 7, 8) in 4 dB step until threshold is crossed; intensity is then reduced in 2 dB step until a second cross over is made. The final threshold is set 1 dB between the last 2 presentations. The 4-2 staircase strategy is adopted only when single cross over of threshold is obtained. The resulting time saving is 30% to 50% at randomly selected stimulus locations. Starting luminance for the first four stimuli locations is 2 dB higher than the normal threshold to reduce examination time. [79] The maximum light sensitivity is 20 dB according to the maximum differential light threshold of normal person. [79]

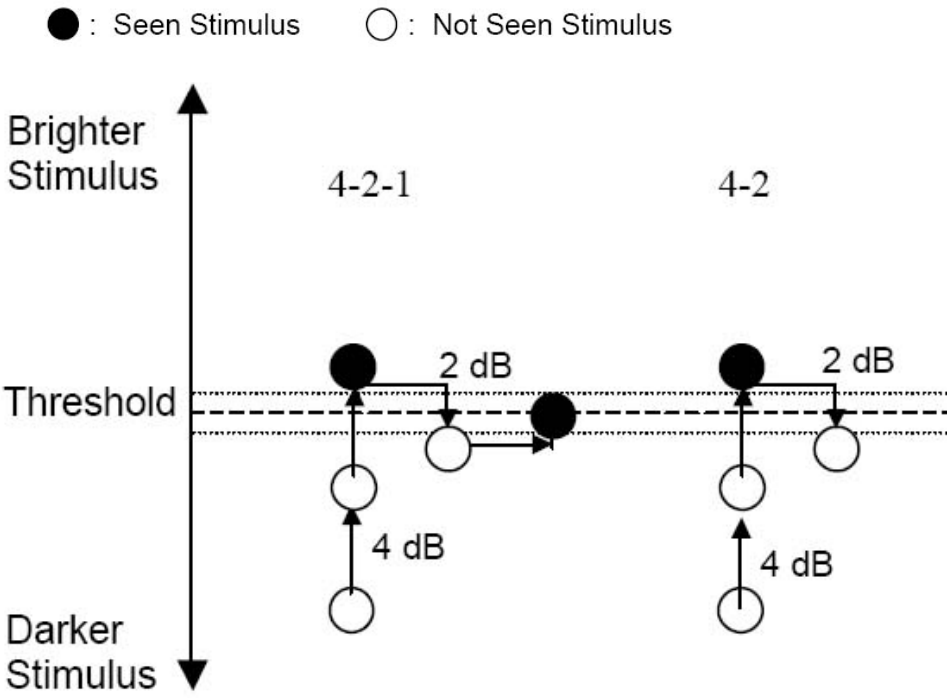

Fig 7. 4-2-1 and 4-2 Strategy with initial stimulus under threshold (Microperimeter MP1 Operator's Manual 2007) [80]

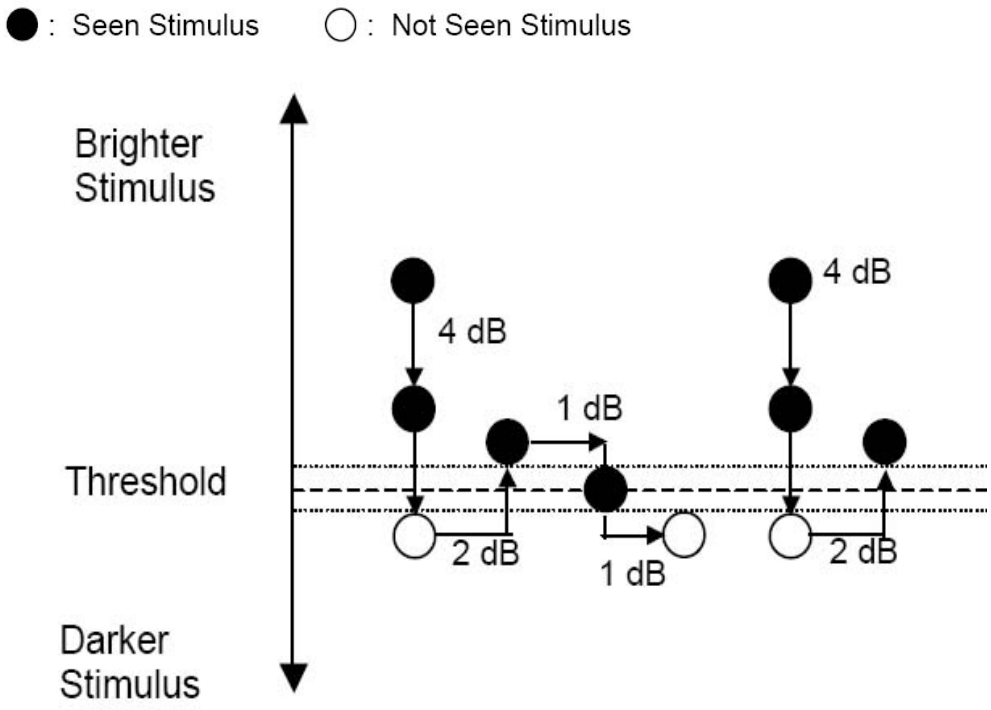

Fig 8. 4-2-1 and 4-2 Strategy with initial stimulus over threshold (Microperimeter MP1 Operator's Manual 2007) [80]

Using the MP1 stability and location of fixation can be tested as an isolated test prior to and or after the perimetric procedure to gain information of the patients compliance. It has to be kept in mind that the accuracy of the initial stored fundus photograph is of major importance when judging the exact location of fixation. [79]

1.10 Purpose of the study

The purpose of this study is to evaluate the anatomical and functional outcome of vitreoretinal microsurgery with internal limiting membrane (ILM) peeling. The influence of the additional use of brilliant blue G staining, of the ILM during surgery should also be examined in eyes suffering from macular hole, macular pucker or vitreomacular traction.

2. PATIENTS AND METHODS

The study was conducted as an observational, non randomized prospective study of forty-eight patients who underwent vitreomacular surgery with internal limiting membrane peeling. Treatment indications were idiopathic macular hole, macular pucker and vitreomacular traction syndrome. The study was carried out at Ludwig-Maximilians University- Eye hospital-Munich, Germany from April 2009 to March 2010. The use of the staining agent BBG during vitreous surgery was at the discretion of the surgeon during the procedure.

Patients were classified into 2 groups:

A- Sixteen patients suffering from idiopathic macular hole, were treated by internal limiting membrane (ILM) peeling following 23g pars plana sutureless vitrectomy. This group was further subdivided into:

1A- Fourteen eyes that underwent ILM peeling assisted by BBG staining.

2A- Two eyes that underwent ILM peeling without assisted staining.

B- Twenty-four patients suffering from primary macular pucker or vitreomacular traction syndrome, were treated by internal limiting membrane (ILM) peeling following 23g pars plana sutureless vitrectomy. This group was further subdivided into:

1B- Seventeen eyes that underwent ILM peeling assisted by BBG staining.

2B- Seven eyes that underwent ILM peeling without assisted staining.

2.1 Preoperative examinations:

2.1.1 Assessment of the best corrected visual acuity and refraction:

The refraction was measured using a Rodenstock autorefractometer; the best-corrected visual acuity testing was done using decimal chart.

2.1.2 Slit lamp examination:

This was performed to assess the condition of the cornea, the crystalline lens, the pupil, the iris and to measure the intraocular pressure by applanation tonometry. Further, indirect

ophthalmoscopy was used under dilated pupil condition (1% tropicamide). The fundus was examined to detect retinal detachment, peripheral breaks or holes, the state of the posterior hyaloid, the condition of the vitreous and the choroid.

2.1.3 Fundus photography:

Photographs of the macular area were taken using Zeiss fundus camera with red free light.

2.1.4 Fundus autofluorescence, fluorescein angiography, infrared images and spectralis OCT (Heidelberg HRA+OCT):

This was done to assess macular thickness, edema, macular pucker, vitreomacular traction and macular hole.

2.1.5 Microperimetry (MP1):

Fundus–monitored Microperimetry was performed through normal pupil in a dark room using MP1 (Nidek Instruments Inc., Padova, Italy). The MP1 software contains an automatic tracking system for fundus movements; this evaluates every acquired frame for shifts in the x and y direction of the fundus with respect to a reference frame obtained by an infrared camera at the beginning of the examination.

The patient was informed to press on a special button connected to the microperimeter as soon as the patient can see a light stimulus. If the patient can see the weakest stimulus (20dB) this means highest macular sensitivity.

A 4-2-1 staircase strategy with Goldmann III stimulus was used and 40 stimulus locations covering the center 10° were examined. The white background illumination was set at 1.27 cd/m^2. The differential luminance, defined as the difference between stimulus luminance and background luminance, was 127 cd/m^2 at 0 dB stimulation and the maximum stimulus attenuation was 20 dB. The duration of the stimulus was 200 milliseconds and the fixation target was varied in size according to the patient's visual acuity. There were eight location spots covering the central 2° field, 24 location spots covering the central 6 ° field, and 40 location spots covering the entire central 10° field. After fundus Microperimetry a colored fundus image was taken, registered within the Microperimetry.

2.2 Exclusion criteria:

Eyes with hereditary retinal diseases, after laser photocoagulation, pathological myopia, inflammatory choroidopathies, retinal detachment, dense vitreous hemorrhages, diabetic retinopathy, age related macular degenerations, ocular trauma or past vitreoretinal surgeries and patients with lens opacity grades 3, 4 and 5 were excluded from the study.

2.3 Operative techniques:

2.3.1 -Anesthesia:

-Local retro-bulbar anesthesia was used in 35 patients, general anesthesia in five patients.

2.3.2 -Surgical procedure:

Combined cataract surgery and sutureless trans-conjunctival 23g pars plana vitrectomy were performed in 19 cases and sutureless pars plana vitrectomy only in 21 cases.

The surgical procedures were done by four vitreoretinal surgeons at the Eye hospital of Ludwig-Maximilians University.

2.3.2.1-Catarct surgery: Using micro incision cataract surgery. [81]

Corneal incision was created, 2.00 mm diameter in size in the upper part, first horizontally, followed vertically to create a valve like opening. Additional 2 side ports were made using superblade 15° at 2 and 10 o'clock. Afterwards injection of a viscoelastic material into the anterior chamber was performed using healon. This was used to stabilize the anterior chamber and to protect the corneal endothelium. In the next step, needle capsulorhexis was performed by using a cystotome that was created from a 27 g bended needle. The anterior capsule was perforated at the center of the crystalline lens; the needle was pulled towards the periphery, tearing the anterior capsule for a length equivalent to 2/3 the radius of the rhexis as desired. A flap of the capsule was raised and progressively pulled in an anti-clockwise direction to produce a continuous circular opening of the anterior capsule. Hydrodissection was performed by injection of a small

amount of a balanced saline solution (BSS) under the capsule until a wave was observed in the pupillary field, propagated to the opposite side between the posterior capsule and the cortex. Hydro-delineation was done by injecting fluid into the nucleus, to help to separate the nucleus from the cortex and epinucleus and to protect the posterior capsule during phacoemulsification. Thereafter, phacoemulsification was done using varying phacovitrectomy machines (Geuder, Oertli or Alcon). Different techniques were used to emulsify the nucleus, for examples 'Divide and Conquer', 'Phaco-Chop', 'Stop and Chop' and 'Quick and Chop' according to the lens status. Finishing Phacoemulsification a switch to bimanual system was done for infusion-aspiration of cortical fragments and to clean the capsular bag from any lens fibers.

After cleaning the capsular bag, again, a viscoelastic material was injected into the anterior chamber (Healon). The wound bed was widened another 0.5 mm to implant an acrylic lens into the capsular bag. The viscoelastic material was aspirated and hydration of the wound bed was then done to close the self sealing wound. [77]

2.3.2.2 -Pars plana vitrectomy: was performed using 23-gauge sutureless pars plana vitrectomy technique.[82] After insertion of 3 trocars a core vitrectomy was done and the posterior hyaloid was detached using a cut rate of 3000 Cuts/Min. Peripheral vitreous was removed to relief any potential peripheral traction and to detect any potential peripheral breaks.

After central vitrectomy, a disposable macular contact lens was applied to the cornea to get good viewing of the macular area. Epimacular membrane and ILM peeling were done either with or without assisted staining of the ILM.

An Eckard ILM forceps was applied near the vascular arcades to grasp the ILM, and to peel the ILM within the vascular arcades and over the foveola. In cases of BBG staining of the ILM after vitrectomy, BBG solution (Brilliant peel, sterile 1 ml ampule, containing 0.25 mg brilliant blue G - Fluoron, Neu-Ulm, Germany) was injected into the vitreous to cover the retina. After a few seconds, the vitreous chamber was washed with BSS and the ILM stained blue. ILM peeling was performed as mentioned above.

At the end of the surgery, the vitreous cavity was filled with air, the saline was removed via a float needle. In cases of macular hole 15% C2F6 gas was injected through the infusion canula to achieve a prolonged tamponading of the macular hole. In macular pucker cases, no fluid air exchange was done and the operation was ended with saline tamponading.

2.4 Postoperative management:

Face down positioning was achieved for all patients after the use of C2F6 15% gas. The others had no special position. Patients were examined daily for 5 days to assess the condition of anterior segment, intra-ocular pressure (IOP) and retinal attachment.

Postoperative medication included combination of antibiotic and corticosteroid eye drops 4 times per day for up to one month with gradual tapering, depending on the condition of the eye.

All the preoperative examinations mentioned before were repeated again at the postoperative follow up (mean 93.875 days).

2.5 Statistics:

Statistics were carried out using Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA) and analyzed using SPSS 17.0 for windows (SPSS Inc.,Chicago, IL USA). The Wilcoxon signed-rank test was used .On all tests $p < 0.05$ was considered significant.

2.6 Ethical issues:

The study was performed in accordance to the ethical standards laid down in the 1964 declaration of Helsinki and Institutional Review Board approval was obtained.

3. RESULTS

Forty patients were enrolled in the study, 22 males and 18 females. The average age of all patients was 67.6 years (range 56-79). The average follow up time was 93.875 days (range 86-101) (Table 1). All cases in the postoperative follow up were pseudophakic. The following results represent the findings of the last examinations of the obtained follow-up.

Table 1: Age of patients and follow up period.

	Age [years]	Follow up [days]
Number of eyes	40	40
Mean	67.6	93.875
Standard deviation	± 6.819	± 4.88
Minimum	56	86
Maximum	79	101

3.1- Idiopathic macular hole (group A)

Sixteen eyes with idiopathic macular holes (grade 2 to 4) were included. Fourteen eyes underwent pars plana vitrectomy and peeling of ILM with assisted (BBG) staining and 2 eyes underwent peeling of ILM without using assisted staining (Table 2). All cases of macular hole achieved successful closure of the hole after the first surgery.

Table 2: ILM peeling of eyes with macular hole.

Number of eyes	Frequency	Percent
Without staining	2	12.5%
With (BBG) staining	14	87.5%
Total number	16	100%

3.1.1 Eyes that underwent ILM peeling using BBG assisted staining (A1).

Measurements of the mean visual acuity in eyes that underwent peeling of ILM with assisted BBG staining tested by decimal chart revealed postoperative improvement of two or more lines in 13 eyes and deteriorated in one eye. This improvement was statistically significant (p=0.019) (Table 3).

Table 3. Comparison between mean preoperative versus postoperative visual acuity in eyes that underwent ILM peeling with assisted BBG staining.

Number of eyes	Mean visual acuity preoperative	Mean visual acuity postoperative	P value
14	0.2	0.50	0.019*

* Statistically significant

Using microperimetry in eyes with macular hole that underwent peeling of ILM with BBG assisted staining, revealed a significant postoperative improvement of the mean macular sensitivity from 11.43 dB preoperative to 14.32 dB postoperative

(improved in 12 eyes out of 14). This improvement was statistically significant (p=0.0228) (Table 4, 5).

The mean macular defect improved as well from -4.20 dB preoperative to -2.70 dB postoperative (improvement in 12 eyes out of 14). This improvement was also statistically significant (p=0.0366) (Table 4, 5).

Measurements of the mean fixation spot revealed change from 65.06% preoperative to 66.78% postoperative. However, this change was statistically not significant (p=0.851) (Table 4, 5).

Table 4. Microperimetry relations between the mean preoperative versus postoperative macular sensitivity, mean macular defect and fixation spot in eyes that underwent peeling with assisted BBG staining.

	Mean macular sensitivity pre	Mean macular sensitivity post	Mean macular defect pre	Mean macular defect post	Fixation spot pre	Fixation spot post
Number of eyes	14	14	14	14	14	14
Mean	11.43	14.32	-4.20	-2.70	65.06%	66.78%
Standard deviation	±3.151	±3.167	±2.230	±1.230	±23.411	±24.813
Minimum	5	8	-14	-11	20%	17%
Maximum	17	19	-2	0	99%	97%
P value	0.0228*		0.0366*		0.851	

* Statistically significant

Table 5: Microperimetry relations between pre- and postoperative macular sensitivity, macular defect and fixation spot in eyes that underwent peeling with BBG assisted staining.

		Number of patients
Mean macular sensitivity (post –pre)	A	2
	B	12
	C	0
	Total	14
Mean macular defect (post –pre)	D	2
	E	12
	F	0
	Total	14
Fixation spot (post –pre)	G	7
	H	7
	I	0
	Total	14

A. Mean macular sensitivity postoperative <Mean macular sensitivity preoperative.
B. Mean macular sensitivity postoperative >Mean macular sensitivity preoperative.
C. Mean macular sensitivity postoperative =Mean macular sensitivity preoperative.
D. Mean macular defect postoperative >Mean macular defect preoperative.
E. Mean macular defect postoperative <Mean macular defect preoperative.
F. Mean macular defect postoperative =Mean macular defect preoperative.
G. Fixation spot postoperative < Fixation spot preoperative.
H. Fixation spot postoperative > Fixation spot preoperative.
I. Fixation spot postoperative = Fixation spot preoperative.

3.1.2- Eyes that underwent ILM peeling without assisted staining (A2).

Two eyes that underwent peeling of ILM without assisted BBG staining, the mean visual acuity, improved from 0.15 preoperative to 0.8 postoperative.

Using microperimetry, the mean macular sensitivity of eyes that underwent peeling without assisted BBG staining improved from 16.00 dB preoperative to 18.00 dB postoperative. The mean macular defect was -3.5 dB preoperative and improved to -1.5 dB postoperative. The mean fixation spot was 53% preoperative improved to 74% postoperative (Table 6).

Table 6: Microperimetry relations between the mean preoperative versus postoperative macular sensitivity, mean macular defect and fixation spot in eyes that underwent peeling without assisted BBG staining.

	Macular sensitivity pre-	Macular sensitivity post-	Macular defect pre-	Macular defect post-	Fixation spot pre-	Fixation spot post-
Number of eyes	2	2	2	2	2	2
Minimum	14	17	-5	-3	45%	69%
Mean	16.00	18.00	-3.5	-1.5	53%	74%
Maximum	18	19	-2	0	61%	79%

3.1.3-Results of spectralis OCT measurements in eyes that underwent ILM peeling with and without assisted BBG staining.

Measurements of the central macular thickness by spectralis OCT, revealed a mean central thickness of 240.42 μm postoperative. No preoperative data could be obtained because of the underlying process of macular hole (Table 7).

Table 7. Postoperative OCT macular thickness, using Heidelberg spectralis OCT, with and without BBG assisted staining.

	OCT postoperative μm
Mean	240.42
Standard deviation	± 32.563
Minimum	190
Maximum	298

Case I. Idiopathic macular hole

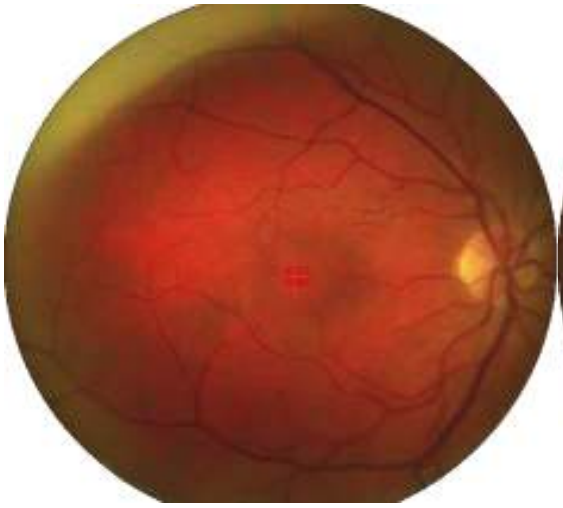

Fig 3-1: Preoperative colored fundus photo of an eye with macular hole stage 3.

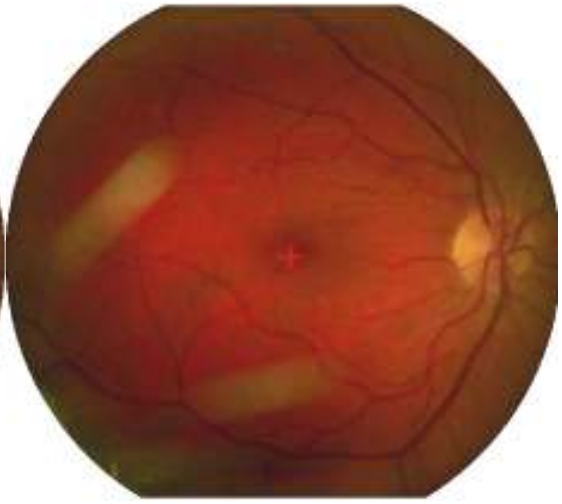

Fig 3-2: Postoperative colored fundus photo of the same eye after hole closure.

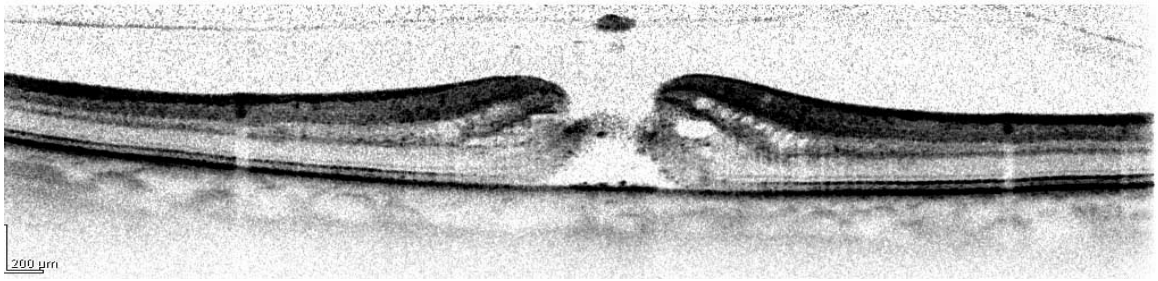

Fig 3-3: Preoperative Spectralis OCT of the macula of the same eye with idiopathic macular hole stage 3.

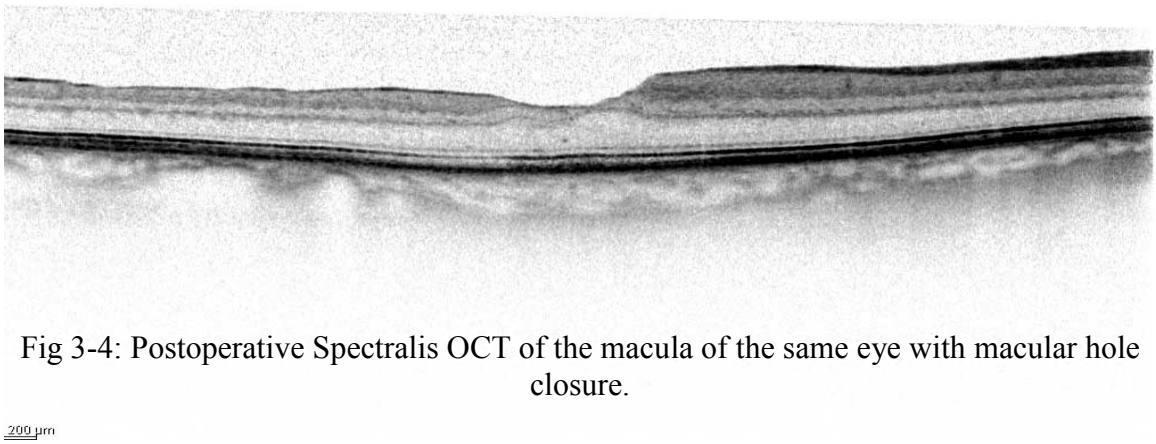

Fig 3-4: Postoperative Spectralis OCT of the macula of the same eye with macular hole closure.

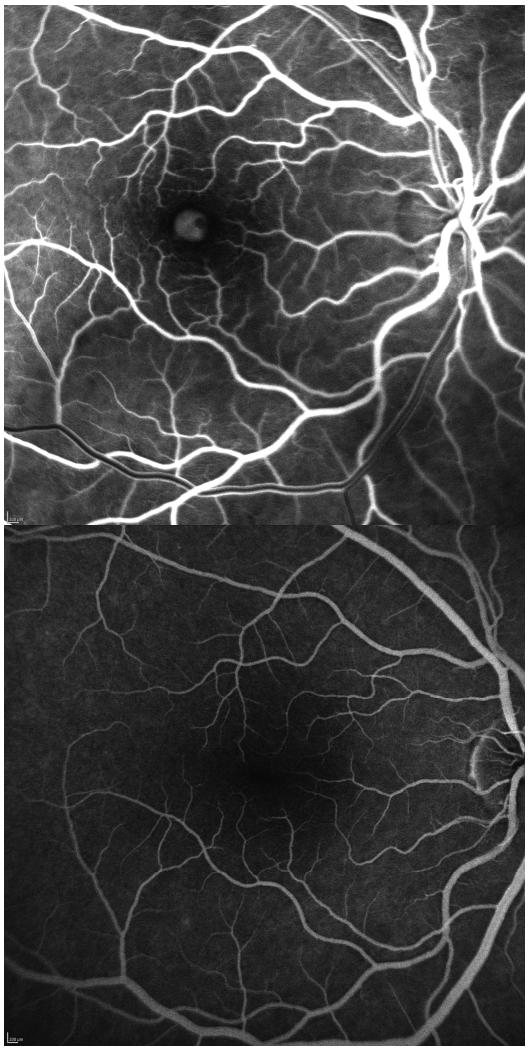

Fig 3-5: Preoperative fundus fluorescein angiography of the same eye with macular hole showing window defect (hyperfluorescence).

Fig 3-6: Postoperative fundus fluorescein angiography of the same eye with macular hole closure (no defect).

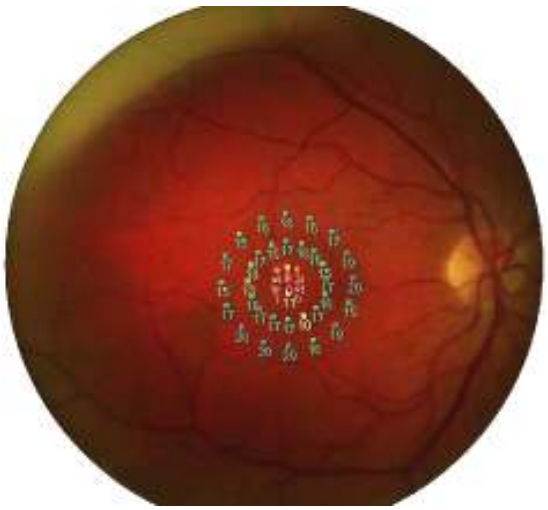

Fig 3-7: Preoperative fundus microperimetry (numerical) of the same eye with macular hole showing central scotoma.

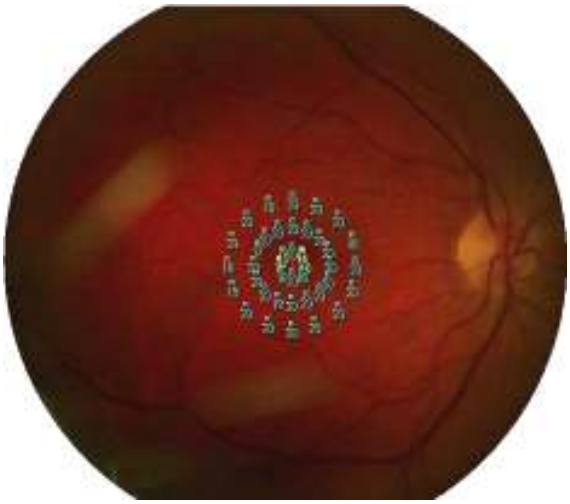

Fig 3-8: Postoperative fundus microperimetry (numerical) of the same eye with macular hole showing no scotoma.

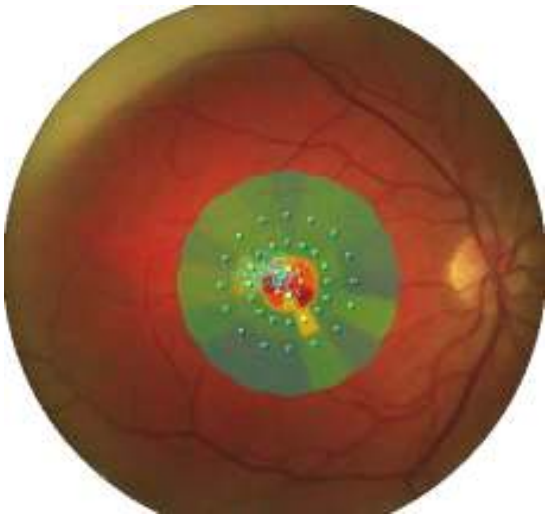

Fig 3-9: Preoperative fundus microperimetry (map) and fixation spot of the same eye with macular hole showing central scotoma and eccentric fixation.

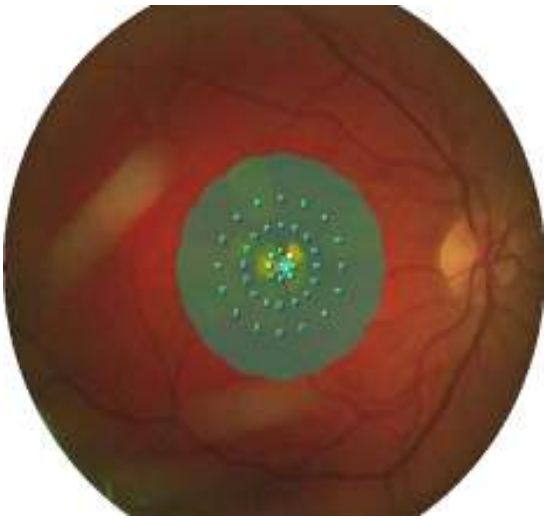

Fig 3-10: Postoperative fundus microperimetry (map) and fixation spot of the same eye after closure of macular hole showing no scotoma and central fixation.

3.2- Primary macular pucker and vitreomacular traction syndrome (group B)

Cases with macular pucker and vitreomacular traction syndrome were studied together as one group because of similarity of their pathogenesis.

Twenty-four eyes with macular pucker and vitreomacular traction were studied, 17 eyes underwent suturless pars plana vitrectomy and peeling of ILM using (BBG) assisted staining and 7 eyes without assisted staining (table 8).

Table 8: ILM peeling of eyes with macular pucker and vitreomacular traction syndrome.

Number of eyes	Frequency	Percent
Without stain	7	29.2%
With (BBG) stain	17	70.8%
Total number	24	100%

3.2.1- Eyes that underwent ILM peeling with assisted BBG staining (B1).

Measurements of the mean visual acuity in eyes that underwent peeling of ILM with assisted BBG staining tested by decimal chart revealed postoperative improvement of two or more lines in 14 eyes, deteriorated in 2 eyes and were equal in 1 eye. This improvement was statistically significant (p=0.02) (Table 9).

Table 9. Comparison between mean preoperative versus post operative visual acuity, in eyes that underwent peeling of ILM with assisted BBG staining.

Number of eyes	Mean visual acuity preoperative	Mean visual acuity postoperative	P value
17	0.35	0.65	0.02*

* Statistically significant

Using spectralis OCT in patients with macular pucker and vitreomacular traction, measuring the central macular thickness of eyes that underwent peeling of ILM with assisted BBG staining revealed non significant postoperative improvement with decrease of the mean central macular thickness from 432.05 µm preoperative to 402 µm postoperative (Table10).

Table 10. OCT measurements of central macular thickness preoperative and postoperative in eyes that underwent peeling of ILM with assisted BBG staining.

	Mean µm	Standard deviation	Minimum	Maximum
OCT pre	432.05	±122	315	740
OCT post	402	±98.510	220	658
P value	0.435			

Using microperimetry in eyes with macular pucker and vitreomacular traction that underwent peeling of ILM with BBG assisted staining revealed improvement of the mean macular sensitivity from 13.95 dB preoperative to 16.30 dB postoperative (improvement

in 14 eyes out of 17). This improvement was statistically significant (p=0.0062) (Table11, 12)

The mean macular defect also improved from -5.72 dB preoperative to -2.88 dB postoperative (improvement in 14 eyes out of 17). This improvement was statistically significant as well (p=0.0367) (Table 11, 12).

Measurements of the mean fixation spot revealed change from 66.32% preoperative to 71.02% postoperative (improvement of 11 eyes out of 17, worsened in 4 eyes and equal in two eyes). However, this change was statistically not significant (p=0.6168) (Table 11, 12).

Table 11. Microperimetry relations between the mean preoperative and postoperative macular sensitivity, mean macular defect and fixation spot in eyes that underwent peeling of ILM with assisted BBG staining.

	Mean macular sensitivity pre-	Mean macular sensitivity post-	Mean macular defect pre-	Mean macular defect post-	Fixation spot pre-	Fixation spot post-
Number of eyes	17	17	17	17	17	17
Mean	13.95	16.30	-5.72	-2.88	66.32%	71.02%
Standard deviation	± 2.112	± 2.540	± 3.743	± 3.851	± 26.350	± 27.856
Minimum	4	8	-12	-10	24%	9%
Maximum	17	18	-4	-1	100%	100%
P value	0.0062*		0.0367*		0.6168	

* Statistically significant

Table 12. Microperimetry relations between pre- and postoperative macular sensitivity, macular defect and fixation spot in eyes that underwent peeling of ILM with BBG assisted staining.

		Number of patients
Mean macular sensitivity (post –pre)	A	2
	B	14
	C	1
	Total	17
Mean macular defect (post –pre)	D	2
	E	14
	F	1
	Total	17
Fixation spot (post –pre)	G	4
	H	11
	I	2
	Total	17

A. Mean macular sensitivity postoperative <Mean macular sensitivity preoperative.
B. Mean macular sensitivity postoperative >Mean macular sensitivity preoperative.
C. Mean macular sensitivity postoperative =Mean macular sensitivity preoperative.
D. Mean macular defect postoperative >Mean macular defect preoperative.
E. Mean macular defect postoperative <Mean macular defect preoperative.
F. Mean macular defect postoperative =Mean macular defect preoperative.
G. Fixation spot postoperative < Fixation spot preoperative.
H. Fixation spot postoperative > Fixation spot preoperative.
I. Fixation spot postoperative = Fixation spot preoperative.

3.2.2- Eyes that underwent ILM peeling without assisted staining (B2).

Measurements of the mean visual acuity of 7 eyes that underwent peeling of ILM without assisted staining tested by decimal chart, revealed postoperative improvement of one or more lines in 6 eyes and deteriorated in one eye. This improvement was statistically not significant (p=0.97) (Table 13).

Table 13. Comparison between mean preoperative and postoperative visual acuity in eyes with macular pucker and vitreomacular traction that underwent peeling of ILM without assisted staining.

Number of eyes	Mean visual acuity preoperative	Mean visual acuity postoperative	P value
7	0.40	0.50	0.97

Using microperimetry in eyes with macular pucker and vitreomacular traction that underwent peeling of ILM without assisted staining revealed improvement of the mean macular sensitivity from 14.52 dB preoperative to 16.6 dB postoperative (improved in 6 eyes out of 7). However, this improvement was not statistically significant (p=0.0928) (Table 14, 15)

The mean macular defect also improved from -4.35 dB preoperative to -3.42 dB postoperative (improvement in 6 eyes out of 7). This improvement was statistically not significant as well (p=0.4609) (Table 14, 15)

Measurements of the mean fixation spot revealed change from 58.94 % preoperative to 72.71% postoperative with improvement of 4 eyes out of 7 and worsened in 3 eyes. This change was statistically not significant (p=0.2996) (Table 14, 15).

Table 14. Microperimetry relations between the mean preoperative versus postoperative macular sensitivity, mean macular defect and fixation spot in eyes that underwent peeling without assisted BBG staining.

	Mean macular sensitivity pre	Mean macular sensitivity post	Mean macular defect pre	Mean macular defect post	Fixation spot pre	Fixation spot post
Number of eyes	7	7	7	7	7	7
Mean	14.52	16.6	-4.35	-3.42	58.94%	72.71%
Standard deviation	± 2.54	± 1.621	± 2.854	± 1.513	± 25.02	± 22.43
Minimum	10	15	-9	-5	22%	39%
Maximum	17	19	-2	-1	92%	98%
P value	0.0928		0.4609		0.2996	

Table 15. Microperimetry relations between the mean preoperative versus postoperative macular sensitivity, mean macular defect and fixation spot in eyes that underwent ILM peeling without assisted BBG staining.

		Number of patients
Mean macular sensitivity (post –pre)	A	1
	B	6
	C	0
	Total	7
Mean macular defect (post –pre)	D	1
	E	6
	F	0
	Total	7
Fixation spot (post –pre)	G	3
	H	4
	I	0
	Total	7

A. Mean macular sensitivity postoperative <Mean macular sensitivity preoperative.
B. Mean macular sensitivity postoperative >Mean macular sensitivity preoperative.
C. Mean macular sensitivity postoperative =Mean macular sensitivity preoperative.
D. Mean macular defect postoperative >Mean macular defect preoperative.
E. Mean macular defect postoperative <Mean macular defect preoperative.
F. Mean macular defect postoperative =Mean macular defect preoperative.
G. Fixation spot postoperative < Fixation spot preoperative.
H. Fixation spot postoperative > Fixation spot preoperative.
I. Fixation spot postoperative = Fixation spot preoperative.

Using spectralis OCT in eyes with macular pucker and vitreomacular traction, that underwent peeling of ILM without assisted staining revealed non significant postoperative improvement, showing reduction of the mean central macular thickness from 414μm preoperative to 370 μm postoperative (Table 16) .

Table 16: OCT measurements of central macular thickness preoperative and postoperative, using Heidelberg spectralis OCT, in eyes that underwent ILM peeling without assisted staining.

	Mean μm	Standard deviation	Minimum	Maximum
OCT pre	414	± 168.210	309	684
OCT post	370	± 140.23	303	650
P value	0.6047			

Case 2 Macular pucker

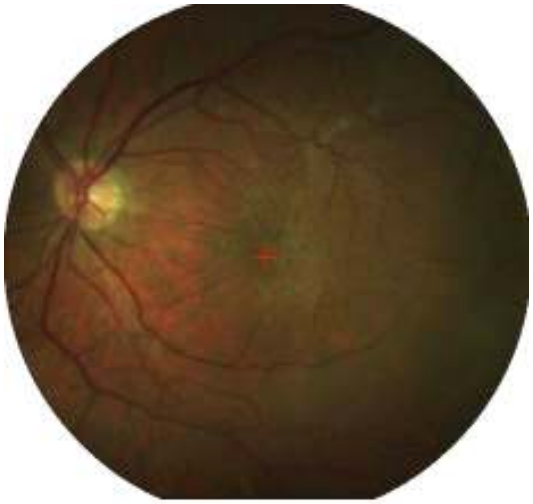

Fig 3-11: Preoperative colored fundus photo of an eye with macular pucker, showing epimacular membrane.

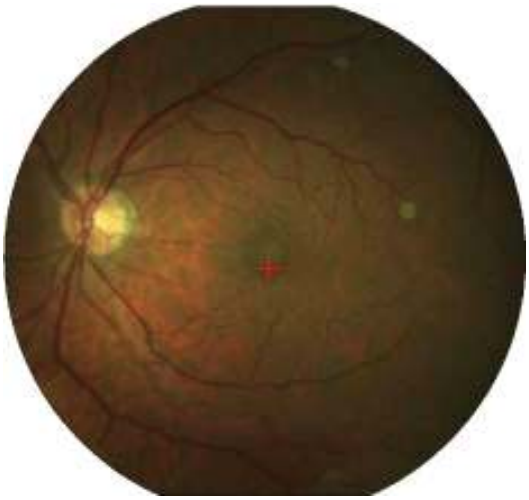

Fig 3-12: Colored fundus photo of the same eye showing postoperative disappearance of epimacular membrane.

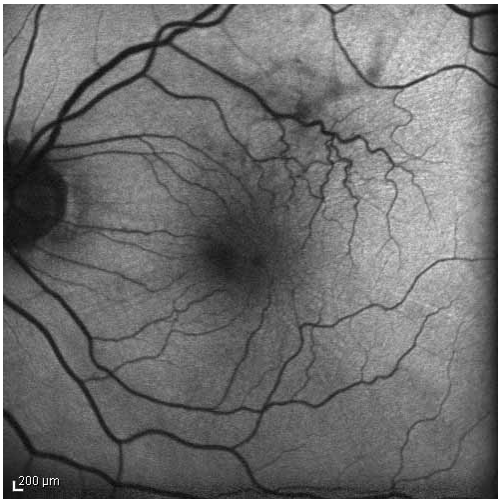

Fig 3-13: Preoperative fundus autofluorescence of the same eye with macular pucker showing epimacular membrane and distortion of blood vessels.

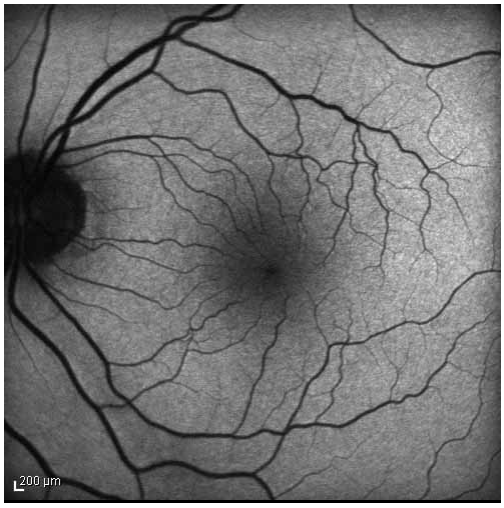

Fig 3-14: Fundus autofluorescence of the same eye in postoperative follow up showing disappearance of epimacular membrane without distortion of blood vessels.

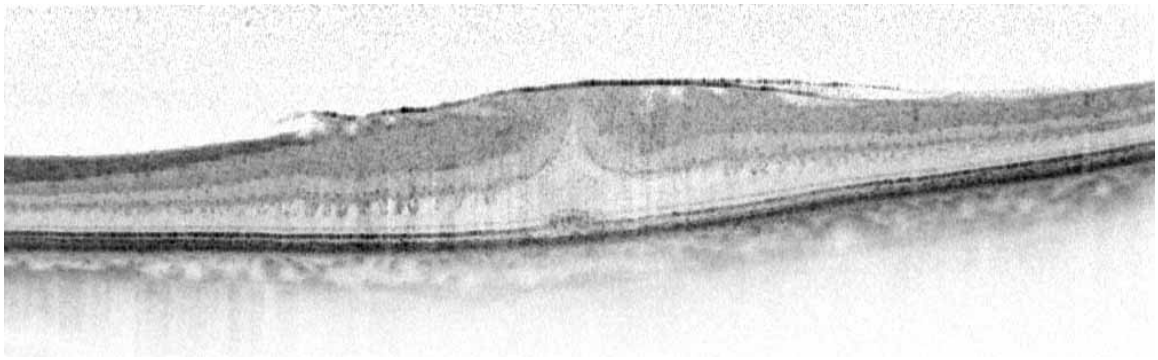

Fig 3-15: Preoperative Spectralis OCT of the macula with macular pucker showing a wrinkled macular surface with an epimacular membrane.

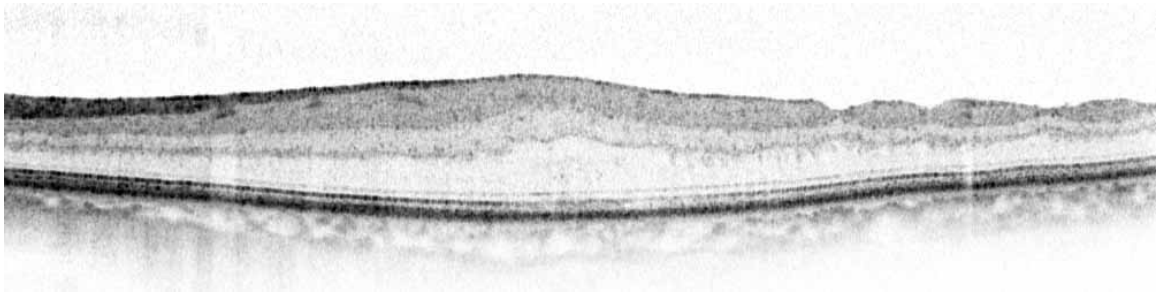

Fig 3-16: Postoperative Spectralis OCT of the same macula (no epimacular membrane visible).

Fig 3-17: Preoperative three dimensions Spectralis OCT of the macula with macular pucker showing wrinkled macular surface and epimacular membrane.

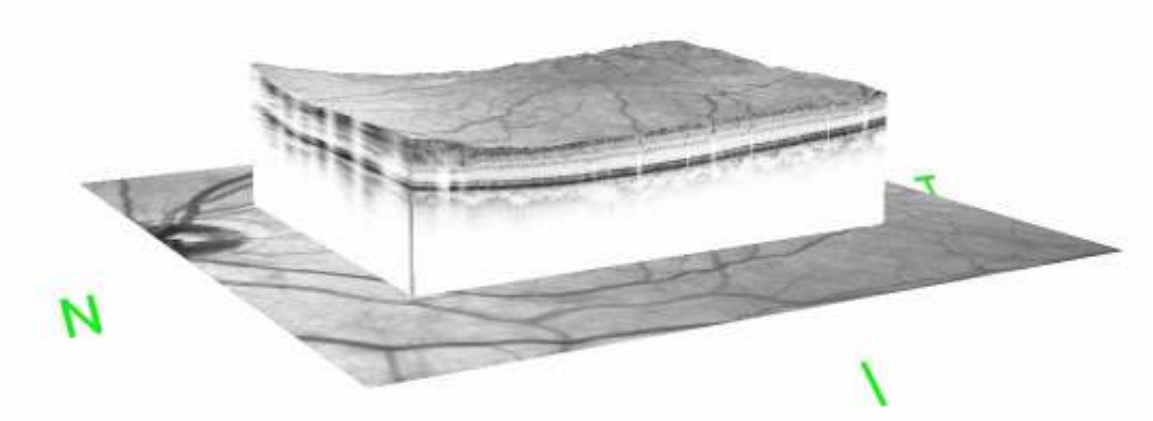

Fig 3-18: Postoperative three dimensions Spectralis OCT of the same macula with macular pucker showing smooth macular surface and disappearance of wrinkles.

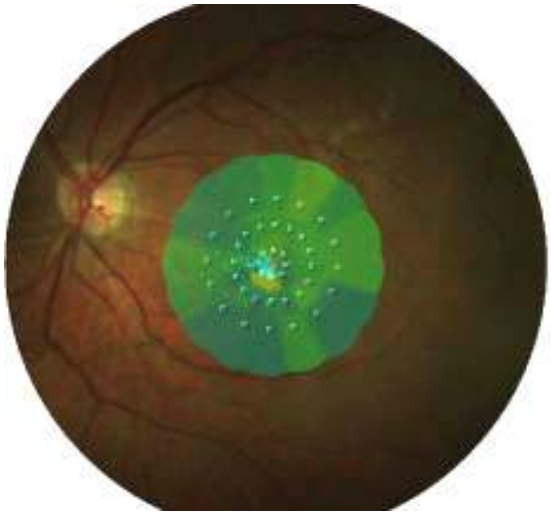

Fig 3-19: Preoperative fundus microperimetry (map) and fixation spot of the same eye with macular Pucker showing decreased sensitivity.

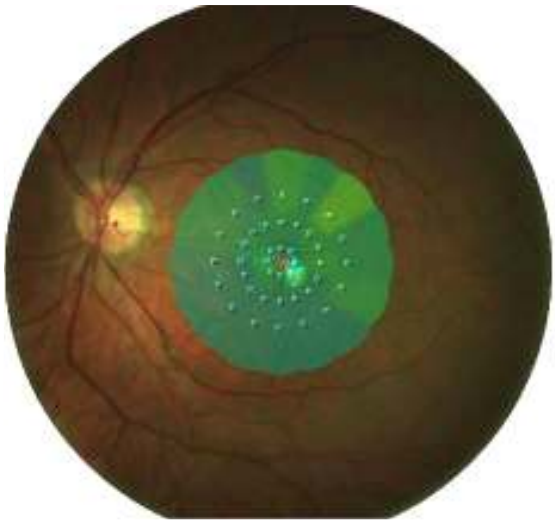

Fig 3-20: Postoperative fundus microperimetry (map) and fixation spot of the same eye with increased sensitivity.

Case 3

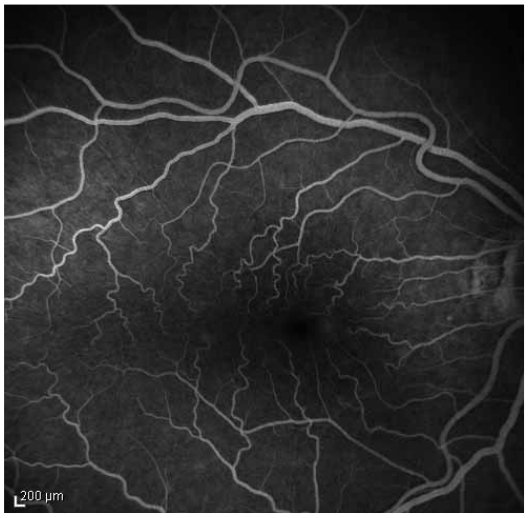

Fig 3-21: Preoperative fundus fluorescein angiography of an eye with macular pucker showing distortion of retinal vessels and mild cystoid edema.

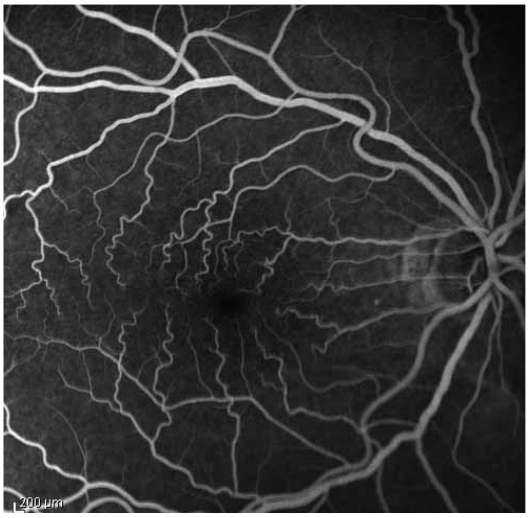

Fig 3-22: Postoperative fundus fluorescein angiography of the same eye with macular pucker showing no distortion of blood vessels and no edema.

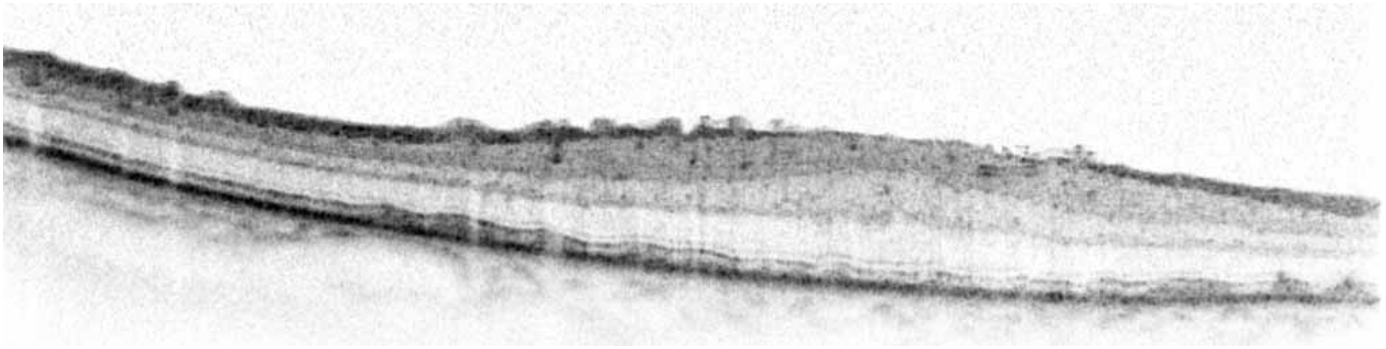

Fig 3-23: Preoperative Spectralis OCT of the macula with macular pucker showing irregular macular surface with wrinkled epimacular membrane.

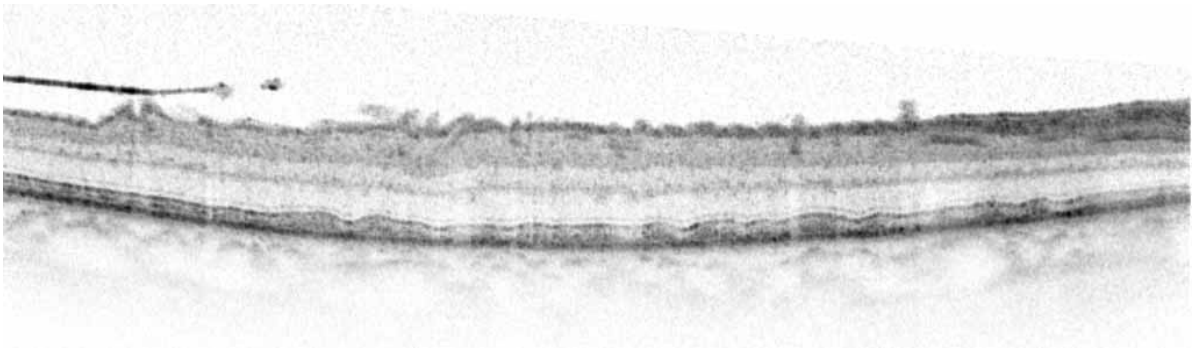

Fig 3-24: Postoperative Spectralis OCT of the same macula with macular pucker showing decreased macular thickness.

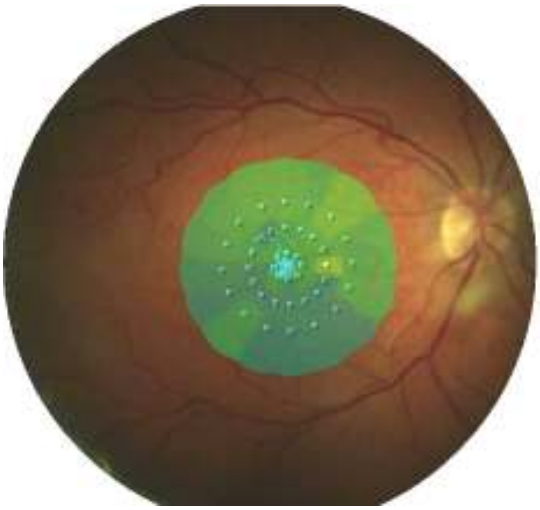

Fig 3-25: Preoperative fundus microperimetry (map) and fixation spot of the same eye with macular Pucker showing decreased sensitivity in the upper part of the macula.

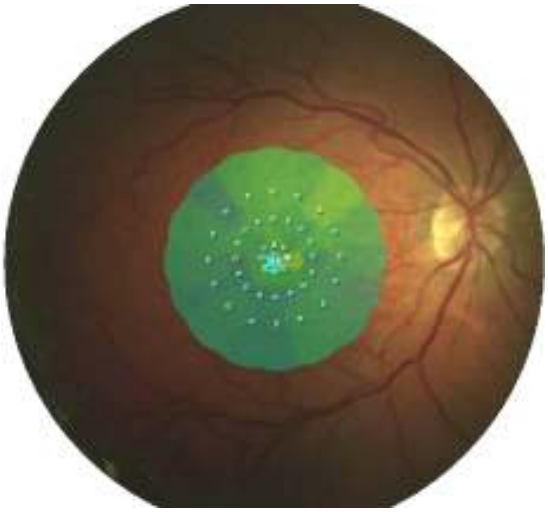

Fig 3-26: Postoperative fundus microperimetry (map) and fixation spot of the same eye with increased sensitivity.

Case 4 Vitreomacular traction

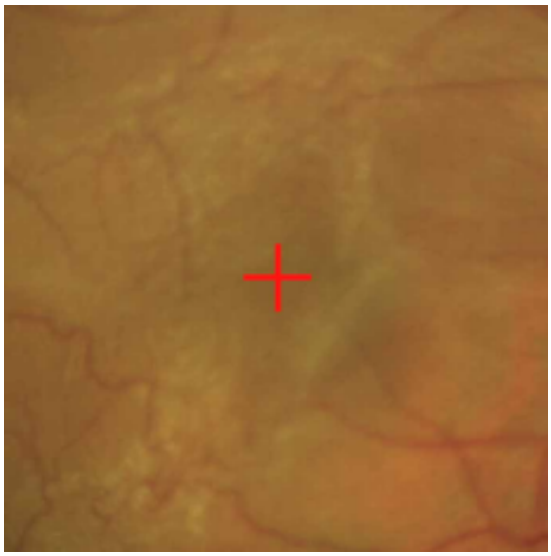

Fig 3-27: Preoperative colored fundus photo of an eye with vitreomacular traction showing tractional membrane.

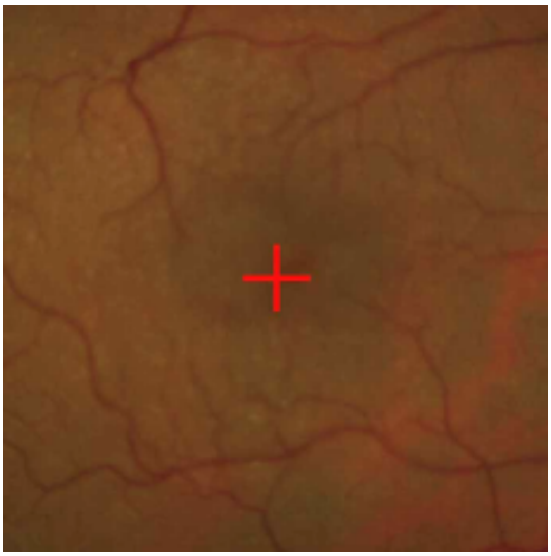

Fig 3-28: Postoperative colored fundus photo of the same eye with vitreomacular traction showing no membrane.

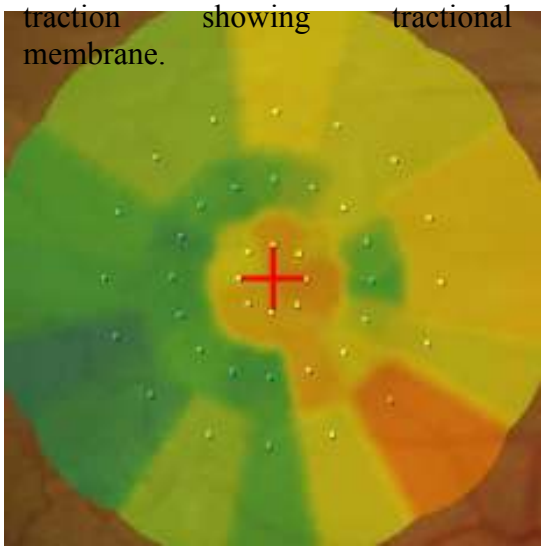

Fig 3-29: Preoperative fundus microperimetry (map) of the same eye with vitreomacular traction showing marked decrease of macular sensitivity.

Fig 3-30: Postoperative fundus microperimetry (map) of the same eye with vitreomacular traction showing improvement of macular sensitivity

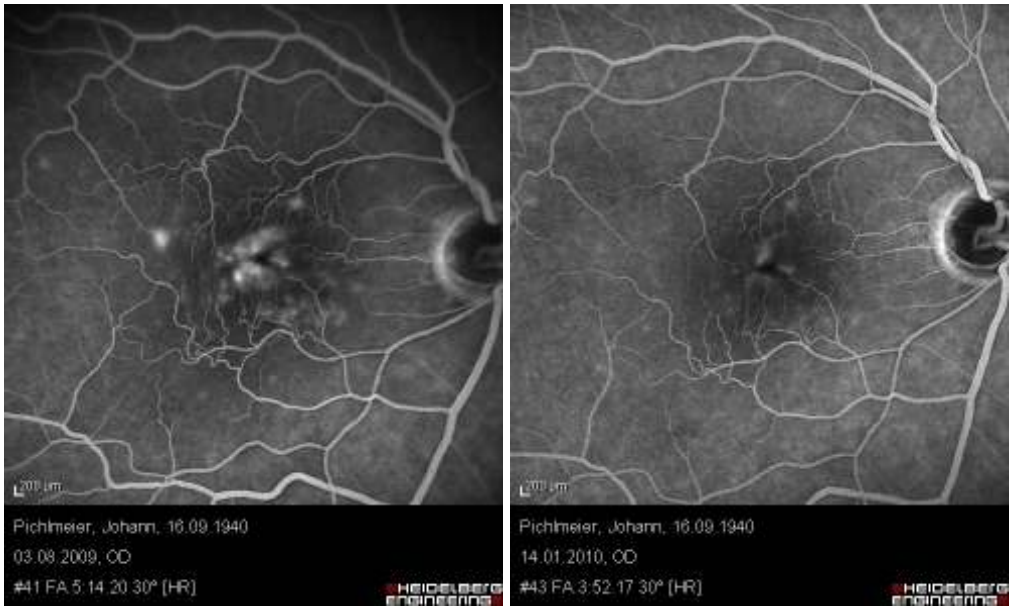

Fig 3-31: Preoperative fundus fluorescein angiography of the same eye with vitreomacular traction showing moderate cystoid macular edema.

Fig 3-32: Postoperative fundus fluorescein angiography of the same eye with vitreomacular traction showing mild cystoid macular edema.

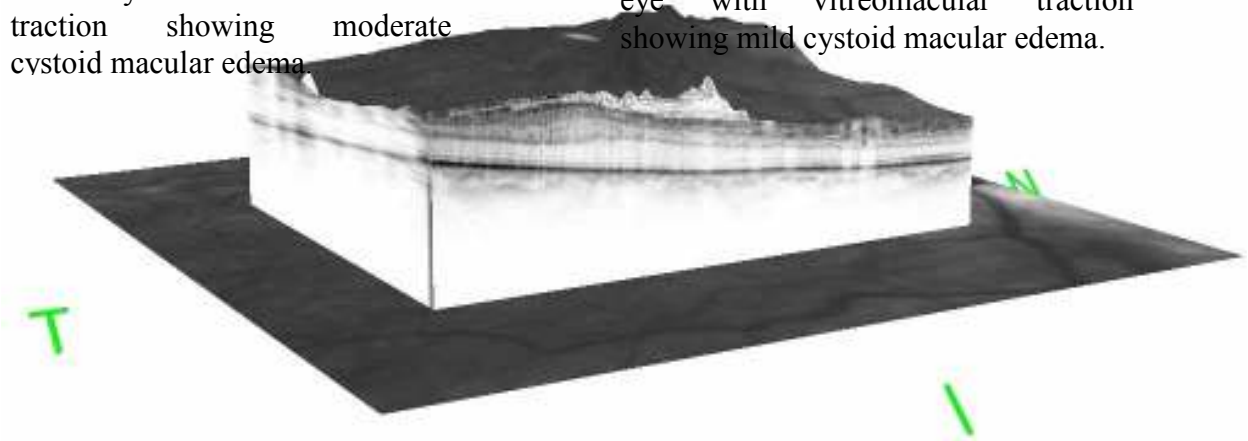

Fig 3-33: Preoperative three dimensions Spectralis OCT of the macula with vitreomacular traction showing marked irregularities of the macular surface with epiretinal membrane.

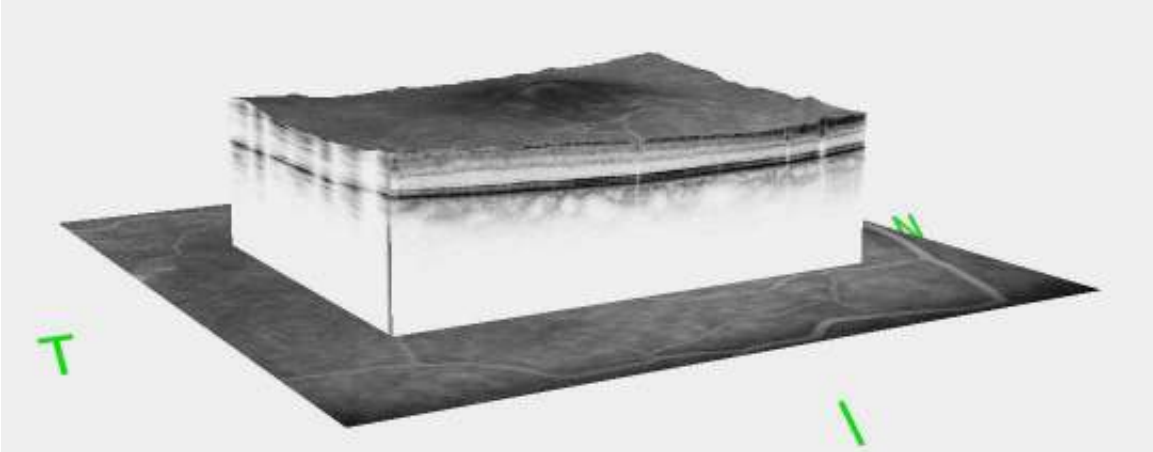

Fig 3-34: Postoperative three dimensions Spectralis OCT of the same macula with vitreomacular traction showing improvement of the surface irregularities.

4. DISCUSSION

Vitreous surgery with epiretinal membrane peeling and removal of the ILM can successfully treat macular hole, vitreomacular traction and macular pucker. However, difficulties in visualization of the virtually translucent ILM can present technical challenges. It is widely recognized that without surgical adjuvants it may be extremely difficult to remove the membranes due to poor visibility of the ILM and epiretinal membranes. Staining of the ILM is therefore one of the major developments in surgery for such vitreoretinal diseases. [83-84]

Brooks (2000) reported that ILM peeling seemed to be especially beneficial in eyes with primary surgical failure or reopened, large or chronic holes and reported that an effect of ILM peeling was most obvious in eyes after primary surgical failure and for holes with longer duration or large size. [46]

Several vitreoretinal publications have discussed the functional and anatomical outcome of using various types of assisted staining of ILM peeling in macular surgery. None of these researches have evaluated the precise postoperative effects of the new stain BBG on macular functions evaluated by microperimetry in macular hole, macular pucker and vitreomacular traction.

Interpretation of our results was therefore matched with other studies using other stains and different surgical techniques.

With microperimetry, it is possible to achieve a complete mapping of central retinal sensitivity and an analysis of fixation behavior. In addition, it is possible to detect macular damage in macular holes and pucker. [85-86]

In the current study, forty cases were enrolled in a prospective observational study with a mean follow up period of 93.875 days. Cases were classified into 2 major groups; group A included 16 eyes with idiopathic macular hole and group B included 24 eyes with macular pucker and vitreomacular traction syndrome.

Group A (cases with idiopathic macular hole) was further subdivided into 2 subgroups; group A1 included 14 eyes that underwent ILM peeling with assisted BBG staining following pars plana vitrectomy and group A2 included 2 eyes that underwent ILM peeling without assisted staining following pars plana vitrectomy.

Measuring visual acuity in eyes with idiopathic macular hole that underwent peeling of ILM with BBG staining, revealed improvement in postoperative follow up compared to preoperative visual acuity (improvement in 13 out of 14 eyes). A statistically significant postoperative improvement of mean macular sensitivity and mean macular defect in 12 out of 14 eyes (measured by microperimetry) was detected, while the change of postoperative fixation spot to preoperative was statistically not significant.

Eyes that underwent peeling of ILM without assisted BBG staining revealed slight improvement of the mean visual acuity, mean macular sensitivity and mean fixation spot, however, statistical correlation or any other conclusions could not be assessed due to the low number of this subgroup.

Kumagai et al., (2004) reported that the closure rate of holes larger than 400 μm was significantly lower than in holes smaller than 400 μm with or without ILM peeling. The closure rate of holes older than 6 months was significantly lower than in holes younger than 6 months. Preoperative visual acuity of the ILM–peeled group with assisted ICG was better than the non-ILM peeled group. Moreover, visual acuity improvement in the non-ILM peeled group tended to be better than in the ILM–peeled group. This result may be related to ICG use. [87]

Christensen (2009) examined the value of ILM peeling in macular hole. His results showed that surgery with ILM peeling is associated with a significantly higher closure rate than surgery without ILM peeling, 95% and 45% respectively. The author concluded that the visual outcomes were not significantly different between eyes without ILM peeling, peeling with ICG and peeling with trypan blue. [88]

Lee et al., (2005) studied two groups of patients with idiopathic macular hole. They studied 14 patients that underwent ILM peeling with assisted ICG stain and 16 patients

underwent ILM peeling with trypan blue assisted staining. There was a significant difference in the postoperative visual acuities in the trypan blue group with better visual acuities than in the indocyanine green group. Hole closure was successful in 91.9% of all patients. [89]

Tognetto et al., (2006) studied patients suffering from macular hole, treated with and without ICG assisted ILM peeling. Anatomic success was more frequent when ICG was not used. In addition patients who achieved hole closure after ILM peeling, the functional results were better when ICG was not used. [90]

Our results were similar in some aspects to Ferencz et al., (2006) who studied two groups of patients with idiopathic macular hole, group A consisted of 21 eyes that underwent peeling with indocyanin green (ICG) assisted staining, group B consisted of 9 eyes that underwent ILM peeling without assisted staining. Visual acuity improved in both groups. There was no statistically significant difference between groups A and B at three and six months after surgery, after 12 months in group B there was a significant improvement in visual acuity compared to baseline. At 20 months follow up examination a significant improvement in both groups compared with the preoperative results was observed, and visual acuity was significantly better in group B than group A. [91]

Figueroa et al., (2008) studied patients with macular hole, the first group of 9 patients were subjected to ILM peeling with trypan blue 0.06 % assisted staining and second group of 9 patients trypan blue 0.15% assisted staining was used. There was no statistically significant difference between postoperative visual acuities of the two groups when different concentrations of trypan blue dye were used. Also in ERG examination, no significant difference was found between the eyes with macular hole and the contralateral eyes before and after surgery. The closure rate was 88.9% for group 1 and 100% of group 2. [92]

In our study, group B (macular pucker and vitreomacular traction syndrome cases) was further subdivided into 2 subgroups; group B1 consisted of 17 eyes that underwent ILM peeling with assisted BBG staining following pars plana vitrectomy and group B2

consisted of 7 eyes that underwent ILM peeling without assisted staining following pars plana vitrectomy. Visual acuity of eyes with assisted staining revealed, significant postoperative improvement compared to preoperative visual acuity.

The central macular thickness of our macular pucker and vitreomacular traction syndrome cases, measured by spectralis OCT, showed obvious postoperative improvement with decrease in mean macular thickness, after vitrectomy and ILM peeling whether with or without staining.

Using microperimetry, the correct diagnosis of macular holes versus pseudo-holes or epiretinal membranes, which may be difficult to differentiate in early stages, is simplified by identification of the amount of functional impairment. Eyes suffering from macular holes develop an absolute scotoma. Consecutively, a movement of fixation outside the pathologic area mostly towards the left margin at the retina i.e. temporal in right eyes. Stability of fixation showed no significant correlation to visual acuity. [93-94]

Using microperimetry in the current study, cases with macular pucker and vitreomacular traction that underwent peeling of ILM with BBG assisted staining revealed significant postoperative improvement of mean macular sensitivity and mean macular defect in 14 out of 17 eyes, while the postoperative change in the fixation spot was statistically not significant.

Measurement of postoperative visual acuity of eyes that underwent peeling of ILM without assisted staining revealed non-significant improvement compared to the preoperative acuity. Similarly, a statistically non-significant improvement of mean macular sensitivity, macular defect and fixation spot was detected in these cases.

Park et al., (2003) studied 44 patients with macular pucker divided into 2 groups. Group 1, consisted of 24 patients with macular pucker that underwent pars plana vitrectomy and ILM peeling without assisted staining. Group 2, consisted of 20 patients that underwent pars plana vitrectomy and removal of epimacular membrane without ILM peeling. Visual acuity improved or was unchanged in 19 of 24 eyes without ILM peeling and in all eyes with ILM peeling. At the final visit 21% of eyes without ILM peeling

showed evidence of recurrent macular pucker or persistent contraction of the ILM and the retinal vessels. None of the eyes with ILM peeling had evidence of a recurrent macular pucker. [45]

Larsson Jörgen (2004) studied 11 patients with vitreomacular traction syndrome that underwent pars plana vitrectomy with posterior hyaloid removal but without ILM peeling. The mean retinal thickness was 608 μm ± 260 μm and improved to 243 μm± 66 μm after 6 months follow up. This difference was statistically significant. [95]

Chung et al., (2008) studied 6 eyes with vitreomacular traction syndrome that underwent pars plana vitrectomy and peeling of posterior hyaloid without ILM peeling. The visual acuity significantly improved from 0.4 to 0.75, the mean preoperative foveal thickness was 406 μm, significantly improved and decreased to 241 μm 3 months after surgery. [96]

On the contrary of our results, Gandorfer et al., (2003) studied 14 patients with epiretinal membranes that underwent peeling of ILM in 5 eyes with assisted ICG peeling and 9 eyes without assisted staining. Preoperative visual acuity ranged from 20/400 - 20/30 equivalent to 0.05 - 0.67 decimal. Final postoperative visual acuity ranged from 20/400-20/20 equivalent to 0.05 - 1 decimal. The postoperative visual acuity improved in 8 eyes, stable in 5 eyes and deteriorated in one eye. In five out of six eyes without postoperative visual improvement, indocyanine green was used to stain ILM. [66]

Li et al., (2003) examined 14 patients with epiretinal membranes that underwent pars plana vitrectomy with internal limiting membrane assisted trypan blue 0.06% peeling. The mean age was 60.7 years with preoperative visual acuity of 1/60 to 6/6 Snellen chart, equivalent to 0.02 1.0 decimal. Postoperative visual acuity improved and ranged from 5/60 to 6/6 Snellen chart, equivalent to 0.083 to 1 decimal, with a mean follow up period of 4.4 months. Vision improved or maintained in all patients. [55]

Haritoglou et al., (2004) studied patients with macular pucker. The first group of 10 patients were subjected to ILM peeling with trypan blue assisted staining and the second

group of 10 patients that underwent ILM peeling, no assisted staining was used. A deterioration of visual acuity was seen only in group 2 (without trypan blue) in 3 of 10 patients. [44]

Our results were similar to Enaida et al., (2006) who examined 10 patients with macular hole and 10 patients with macular pucker. All cases underwent ILM peeling, using BBG assisted staining. Macular holes were closed anatomically in all cases with postoperative improvement in visual acuity. In macular pucker eyes, the mean central foveal thickness also improved and decreased from 454.7µm preoperative to 249.4 µm postoperative, the mean visual acuity improved postoperatively as well. [97]

Richter-Mueksch et al., (2007) studied 19 patients with idiopathic macular hole and 18 patients with macular pucker that underwent ICG assisted ILM peeling. Microperimetry was done to all cases before surgery and 3 months after surgery. In eyes with macular hole, the mean preoperative macular sensitivity done by microperimetry was 11.3 dB improved to 12.8 dB after 3 months, in eyes with macular pucker the mean preoperative sensitivity was 10.7 dB and improved to 12.7 dB. This increase was statistically significant. Stability of fixation improved and increased significantly in both groups. [86]

Cappello et al., (2009) studied 18 patients with idiopathic macular hole and 41 eyes with macular pucker that underwent ILM peeling with ICG assisted staining, trypan blue assisted staining and without staining. The mean preoperative macular sensitivity (measured by microperimetry) in eyes with macular hole was 10.2dB improved to 12.9 dB at 3 months post operative, 15.0 dB at 6 months and 14.1 dB at 12 months postoperative. In patients with macular pucker the mean preoperative macular sensitivity was 13.7dB and improved to 14.8 dB after 3 months postoperative, 15.1 dB at 6 months postoperative and 15.0 dB at 12 months postoperative. There was no statistical difference in macular sensitivity between eyes using ICG, Trypan blue or without staining that underwent ILM peeling. [98]

Our results were similar in some aspects to Henrich et al., (2009) who studied 17 patients with macular holes, epiretinal membranes, vitreoretinal traction syndromes and cystoid macular edema. All were subjected to peeling of ILM with BBG assisted staining and follow up period of 3 months. The visual acuity increased from 0.25 preoperatively to 0.4 postoperatively. Central retinal OCT thickness showed postoperative improvement with reduction, with range from +7 to -295 μm. [99]

Schumann et al., (2009) examined the retinal cleavage plane of the internal limiting membrane (ILM) removed during macular surgery. In cases that underwent ILM peeling with ICG assisted staining and without staining, a serious amount of inner retinal elements adherent to the retinal side of ILM was detected. It remains uncertain if the presence of retinal cell fragments at the ILM correlates with functional deficit. In contrast, ILM specimens with brilliant blue G staining and trypan blue showed significantly less retinal fragments at the ILM. [100]

In conclusion, we clearly demonstrated that assisted BBG staining of ILM during vitrectomy of eyes suffering from macular hole, macular pucker and vitreomacular traction proved safety of the stain on retinal tissue with postoperative improvement of the acuity of vision, macular thickness, macular sensitivity, macular defect, fixation spot and successful closure of macular hole.

Our results imply that, microperimetry is a safe, non-invasive and accurate documentary investigation that should be done to all macular disease patients especially those undergoing macular surgery pre and postoperative. It can relate the degree of macular dysfunctions "even asymptomatic lesions" to specific anatomical locations.

5. SUMMARY

Purpose: To evaluate anatomical and functional outcome of internal limiting membrane (ILM) peeling with and without BBG staining in cases of idiopathic macular hole, macular pucker and vitreomacular traction syndrome.

Design: Prospective, observational and non randomized study.

Patients and methods: Sixteen eyes suffered from idiopathic macular hole; fourteen eyes underwent pars plana vitrectomy with ILM peeling using BBG staining and two eyes without staining.

Twenty-four eyes suffered from macular pucker and vitreomacular traction syndrome; seventeen eyes underwent pars plana vitrectomy with ILM peeling using BBG staining and seven eyes without staining.

In all patients best corrected visual acuity using decimal chart, spectralis OCT and macular sensitivity (mean sensitivity, mean defect and fixation spot) using microperimetry were determined before surgery and 3 months after surgery.

Results: All cases of macular hole achieved successful closure of the hole after surgery. Measurements of the visual acuity, macular sensitivity and macular defect of eyes with macular hole that underwent peeling of ILM with and without assisted staining, revealed a statistically significant postoperative improvement.

Eyes with macular pucker and vitreomacular traction that underwent peeling of ILM using BBG assisted staining achieved a significant postoperative improvement of visual acuity ($p=0.02$), while a non-significant postoperative improvement occurred in visual acuity of eyes that underwent peeling without assisted staining ($p=0.97$).

Using microperimetry, a statistically significant improvement of the mean macular sensitivity and macular defect was observed in eyes that underwent peeling with assisted BBG staining ($p=0.0062$), ($p=0.\ 0.0367$) respectively and a non-significant improvement of eyes that underwent peeling without assisted staining ($p=0.0928$).

Conclusions: Peeling of ILM is a surgical procedure that can be technically challenging unless a dye is used to stain the transparent ILM.

Assisted BBG staining of ILM during vitrectomy of eyes with macular hole, macular pucker and vitreomacular traction proved safety of the stain on retinal tissue with postoperative improvement of the visual acuity, macular thickness, macular sensitivity,

macular defect and fixation spot. Microperimetry is a safe, non-invasive and accurate documentary investigation that should be done to all macular disease patients especially those undergoing macular surgery.

6. Zusammenfassung

Ziel: Die vorgelegte Arbeit untersucht die anatomischen und funktionellen Ergebnisse nach ILM Peeling, mit oder ohne den Einsatz unterstützender Farbstoffe für die Vitrektomie bei Patienten mit Makulaforamen, Macular pucker und Vitreomakuläres Traktionssyndrom (VMTS).

Design: Prospektive, nicht randomisierte Beobachtungsstudie.

Patienten und Methoden: Von sechzehn Patienten mit idiopathischem Makulaforamen, die mittels pars plana Vitrektomie (ppV) und ILM Peeling behandelt wurden erhielten vierzehn Augen während des Membran Peelings Brilliantblau als Vitalfarbstoff und zwei Augen keinen Vitalfarbstoff.

Von vierundzwanzig Patienten mit Macular pucker und Vitreomakulärem Traktionssyndrom (VMTS), die mittel pars plana Vitrektomie (ppV) und ILM Peeling behandelt wurden erhielten siebzehn Augen während des Membran Peelings Brilliantblau als Vitalfarbstoff und sieben Augen kein Vitalfarbstoff.

Bei allen Patienten wurden die zentrale Sehschärfe (Dezimal-Tafel), eine Spectraldomain OCT und eine Mikroperimetrie zur Beurteilung der Empfindlichkeit, der Defekttiefe der Makula und des Fixationspunktes durchgeführt.

Alle Untersuchungen wurden vor und 3 Monate nach der Operation durchgeführt.

Ergebnisse: In allen Fällen konnte durch die Operation ein Verschluss des Makulaforamens erzielt werden.

Die Bestimmung der Sehschärfe, der Makula-Sensitivität, der zentralen Defekttiefe und des Fixationspunktes der Augen mit Makulaforamen, die einem ILM Peeling mit und ohne Farbstoff unterzogen wurden, ergab bei allen Augen eine postoperative Befundverbesserung. Diese Verbesserung war statistisch signifikant.

Bei den Augen mit Makular Pucker und VMTS, die einer Vitrektomie mit Brilliant Blau G (BBG) unterstütztem Membran Peeling der ILM unterzogen wurden, zeigte sich bei allen eine hoch signifikante postoperative Verbesserung der Sehschärfe (P=0.02), während bei den Augen, die einer Vitrektomie ohne Vitalfarbstoff unterstütztem

Membran Peeling der ILM unterzogen wurden nur eine nicht-signifikante postoperative Verbesserung der Sehschärfe nachgewiesen werden konnte (p=0.97).

In der Mikroperimetrie konnte bei den Augen, die sich einem Membran Peeling mit BBG Unterstützung unterzogen, eine statistisch signifikante Verbesserung der mittleren Makula-Sensitivität (p=0.0062) und der Makula-Defekttiefe (p=0.0367) gezeigt werden. Bei den Augen, die einem Peeling ohne Vitalfarbstoff-Unterstützung unterzogen wurden, konnte hingegen keine signifikante Verbesserung detektiert werden (p=0.0928).

Schlussfolgerungen: Das Peeling der ILM ist ein chirurgisches Verfahren, das technisch anspruchsvoll ist, solange kein Farbstoff verwendet wird.

Die Anwendung von BBG zur Färbung der ILM in Augen mit Makulaforamen, Macular Pucker und Vitreomakuläres Traktionssyndrom (VMTS) erwies sich als sicher mit postoperativer Verbesserung der Sehschärfe, der Makula-Dicke, der Makula-Sensitivität, der Makula- Defekttiefe und des Fixationspunktes.

Die Mikroperimetrie ist eine sichere, nicht invasive und exakte Dokumentations-untersuchung, der sich alle Patienten mit einer Makulapathologie und speziell vor Makulachirurgie unterziehen sollten.

7. REFERENCES

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9. Curriculum vitae

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Education:

High School: Graduated at 1994, general score 95% excellent.
Bachelor: M.B.B.CH (Bachelor of Medicine and Surgery) Faculty of Medicine, Tanta University, Egypt (six years) graduated at October 2000. General score; Excellent.
Postgraduate Studies:
• Master degree in ophthalmology April 2005 (General score: Excellent).
• Ophthalmology Fellowship, Eye hospital, Ludwig-Maximilians-University, Munich, Germany. Since december 2008 until now.

Professional Affiliations:

- Member of the Egyptian Syndicate for Practicing Physicians since 2001.
- Egyptian license for practicing physicians since 2001.
- Member of the Egyptian Ophthalmology society since 2002.

Scientific and working experience:

1- Medical trainee for one year in Tanta University Hospitals 2000-2001.
2- Resident of ophthalmology, Tanta University Hospitals for three years, 2002-2005.
3-Ophthalmology teaching assistant, Faculty of medicine, Tanta University, Egypt since 2005 until now.
4- Ophthalmology Fellowship, Eye hospital, Ludwig-Maximilians-University, Munich, Germany. Since december 2008 until now.
5- Member of Association for Research in Vision and Ophthalmology (ARVO) USA 2010.
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