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Subthreshold Micropulse Diode Laser in Acute Central Serous Chorioretinopathy (ACSCR)

Dr. Basudeb Ghosh, Dr. Anisha Seth, Dr. Richa, Dr. Supriya Arora

Idiopathic central serous chorioretinopathy (CSC) is a disease of neurosensory retinal detachment at the posterior pole. The disease process may be acute, recurrent or chronic. CSC is usually self-limiting and most of the cases resolve in 3-4 months but despite recovery many complain of impaired colour vision, relative scotomas, micropsia, metamorphopsia, decreased contrast sensitivity and nyctalopia in the affected eye. Traditionally treatment has been offered in chronic or recurrent cases. However, recent studies indicate that fluid under the macula for even short durations can lead to permanent photoreceptor damage. Treatment at the acute stage of the disease targets faster visual rehabilitation, restored quality of vision by prompt resolution of the neurosensory detachment (NSD). With the advent of subthreshold lasers there is reduced risk of scarring/ anatomical damage to the retina. The increased safety profile of subthreshold laser allows us to attempt treatment in the acute stage. We did this study to evaluate the effect of Sub-threshold micropulse diode (SMD) Laser therapy laser on acute CSC.

MATERIALS AND METHODS

This was a prospective, interventional case series on 10 eyes of 10 consecutive patients. Patients with first episode of acute CSC presenting within the first 2 months with documented sub-retinal fluid involving the macula and single leak (juxtapveal / sub foveal or extra foveal) on fundus fluorescein angiography (FFA) were included in this study. Patients with evidence of any concomitant ocular disease or trauma, intraocular surgery or laser treatment were excluded. Patients with any history of steroid use or adverse reaction to steroids were also excluded. All patients underwent complete systemic and ocular examination including Best Corrected Visual Acuity (BCVA, Snellen’s chart), Contrast sensitivity (Functional acuity contrast test chart), fundus examination including slit lamp biomicroscopy using 90D and indirect ophthalmoscopy. Investigations included Spectral Domain Optical coherence tomography (RTVue SD-OCT system), Fundus Fluorescein Angiography (VISUCAM 500, Carl Zeiss) and Photostress test.
(PST). Patients were treated using an 810-nm infrared diode laser (OcuLight SLx) as per technique described by Koss MJ et al. A test burn was performed in the retina nasal mid periphery to determine the individual threshold power needed for a visible tissue reaction. The individualized test burn was performed with 125 μm spot, 200 ms exposure duration, and adjusting the power upward in the continuous wave (CW) emission mode until a light grayish visible area is noticed.

Once the threshold CW power (PCW) was determined, the laser was then switched from CW into the Micropulse emission mode set at 15% duty cycle (0.3 ms ‘ON’ time + 1.7 ms ‘OFF’ time=2.0 ms period). The power was doubled (2 x PCW) with the same 200-ms exposure duration delivering a train of 100 Micropulses on and around the area of leak. If at any time, during the treatment, retinal whitening was noted, treatment was immediately stopped.

**RESULTS**

The mean BCVA improved from 0.18 to 0.6 in 2 weeks (p=0.01) and 1.0 at 1 month and 3 months (p<0.001). 2 eyes had resolution of NSD and 7 had decreased NSD at 2 weeks. 8 eyes had complete resolution of NSD and 2 had decreased NSD at 4 weeks. All eyes had complete resolution of NSD at 3 months. The mean PST increased from 35 sec to 17 s at 2 weeks and 10.4s at 4 weeks. PST increased to 7 sec at 3 months (p=0.05). The mean CMT increased from 566 μ to 438.33 μ at 2 weeks and 275 μ at 1 month. It increased to 243 μ at 3 months (p=0.01). All eyes had significant increase in contrast sensitivity.

**DISCUSSION**

A greater understanding of the pathogenesis of central serous chorioretinopathy has led many to believe that CSC is not a completely benign condition. Traditionally treatment is offered to patients with chronic CSC (single episode of greater than 3 months duration with angiographic identification of areas of active leaking site and OCT evidence of serous retinal detachment involving macular region or non-resolving sub-retinal fluid) or recurrent CSC, but the contrast sensitivity and night vision may be permanently impaired in chronic central serous chorioretinopathy.

Recent studies have shown that fluid accumulation under the retina in the acute stage may lead to photoreceptor damage affecting overall quality of vision. This damage persists even after the fluid is reabsorbed spontaneously and may result in diminished contrast sensitivity, which can be permanent, and leave the patient visually impaired. A recent study by Behnia et al., showed that the Subthreshold argon laser treated Acute CSC group had
statistically significant improvement in BCVA and CS at 6 months when compared to the no treatment group. Treatment also resulted in quicker visual recovery. Even when the ultimate BCVA achieved was similar in the two groups, reduced contrast sensitivity was a common sequel when no treatment was given.

SMD Laser therapy improves the metabolism of Retinal Pigment Epithelium (RPE) cells and therefore, accelerates resorption of sub-retinal fluid which in turn leads to less photoreceptor damage without the adverse effects associated with conventional treatment with Argon laser like secondary choroidal neovascularization, scarring on the macula, expansion of the scar to the fovea, and central or paracentral scotomas.\(^5,6\) However one limitation of SMD laser therapy is the absence of an ophthalmologically visible end point.

**CONCLUSION**

SMD Laser therapy lead to faster resolution of NSD with early and complete visual recovery without any side effects of scarring or recurrence in our follow up period.

However, a comparative study with more subjects recruited with a longer duration of follow up is required to further validate these findings.

**REFERENCES**


Sulfurhexafluoride (ST6) Versus Perfluoropropane (C$_3$F$_8$) Gas as Tamponade in Macular Hole Surgery

Dr. Aditya Modi, Dr. Giridhar A, Dr. Mahesh G, Dr. Rameez Najamul Hussain

Original technique for macular hole surgery has been continuously modified and various combinations of gas types and mixtures with different positioning regimens have been reported generally with anatomical success rates of over 95%. In most of these studies, longer-lasting gas mixtures such as perfluoroethane (C$_2$F$_6$) and perfluoropropane (C$_3$F$_8$) have been used that can remain in the vitreous cavity for up to 4 and 8 weeks respectively. Though longer-lasting mixtures of perfluoropropane (C$_3$F$_8$) have shown good anatomical and functional success due to their extensive tamponade effect, they come with the disadvantage of causing impairment of vision for a longer duration, which compromises a patient’s return to daily activities such as driving and prohibits air travel for as long as 8 weeks. This has led to investigations into the use of shorter-acting gas mixtures such as sulfur hexafluoride (SF6) with or without posturing to determine their efficacy and tolerability. Though there are quite a few comparative studies between two gaseous endotamponade agents, small sample size and variable outcome measures were the limitations observed. The present study attempts to compare various outcomes of SF6 versus C$_3$F$_8$ tamponade in surgery for idiopathic macular hole repair in a large sample size with an attempt to study associated complications and also outcome of resurgeries in the same cohort.

MATERIALS AND METHODS

This was a retrospective comparative cohort study carried out in our institute. The study protocol was approved by the institutional ethics committee. Patients who underwent surgery for idiopathic macular hole, between February 2011 and January 2014, with minimum follow up of 3 months were included in the study. 13% C$_3$F$_8$ tamponade was utilised in the patients in the initial half of the study period (referred to as group 2) while the comparative cohort received 20% SF6 as endotamponade agent in the latter half of the study period (referred to as group 1). All patients with secondary macular hole, patients who previously underwent any macular surgery, or patients with any retinal pathology other than macular hole were excluded from the study. All patients were matched for age, gender, laterality, staging, preoperative lens status and duration of symptoms. Preoperative data obtained for each patient included age, gender, duration of symptoms (from onset of symptoms till date of surgery), best corrected
snellen visual acuity which was converted to the negative logarithm of minimal angle resolution (logMAR) for statistical analysis and comparison, intraocular pressure by applanation tonometry, lens status by slit lamp biomicroscopic examination and optical coherence tomography (Spectral domain OCT-Heidelberg engineering, Germany) based macular hole staging. Subsequent intraoperative assessment included careful documentation of any intraocular complication on table with the instrumentation (20/23 gauge) and the gas used as the tamponading agent.

Standard surgical technique was followed for all patients which involved a standard 3-port, 20/23-gauge pars plana vitrectomy with triamcinolone assisted posterior vitreous detachment (PVD) induction. Staining of ILM with diluted brilliant blue was performed for all cases. Dye was left inside for 2 minutes, following which the excess dye was aspirated from the vitreous. Fine forceps were used to peel the internal limiting membrane (ILM) in all cases. After ILM peeling and examining the peripheral retina, a complete fluid air exchange was performed and 20% SF6 or 13% C3F8 gas was injected. All patients were instructed to maintain prone positioning by standard protocol followed in the institution which includes 45 minutes of prone positioning followed by 15 minutes of back rest throughout the day with prone or right/left lateral position during sleeping hours.

Uniform protocol was followed for postoperative review at 1 week, 1 month, 3 months and 6 months with subsequent 6 monthly review till the last date of follow up or whenever clinically indicated. At each visit, best corrected visual acuity, intraocular pressure, lens status, intraocular complications if any, anatomical status of macular hole (OCT based) with resurgery details (if any) were documented.

Cataract progression and strict monitoring of intraocular pressure was done. Development of vision-impairing cataract (which necessitated surgery within 6 months of macular hole repair) were evaluated. Occurrence of glaucoma and its progression was strictly monitored. Optical coherence tomography based monitoring of macular hole status was done with documentation of any persistent or recurrent hole. Same pattern of examination and procedure was followed in case of resurgeries with careful documentation of all details.

The primary outcome measure was anatomical hole closure with secondary outcome measures being changes in best corrected visual acuity, lens status and intraocular pressure.

Wilcoxon signed rank test was used to compare pre and postoperative continuous variables which did not follow normal distribution while Manwhitney U test was used to compare continuous variables of the 2 groups.
which did not follow normal distribution. Categorical data was evaluated using Chi square test and fisher exact test. Proportional test was used to compare data with small sample size. SPSS software Version 16.0 (IBM corp.) was used for statistical analysis.

RESULTS

177 eyes of 166 patients were included in the study. 67 eyes (group 1) had tamponade with SF6 gas, while 111 eyes (group 2) were treated with C3F8 gas tamponade. There was no statistical difference between the 2 groups with regard to age, gender, laterality, optical coherence tomography based staging, intraocular pressure and lens status. Both the groups were similar with regard to intraoperative technique employed (20/23 guage vitrectomy) and dye used (Table 1). Standard postoperative positioning was employed in all cases of both the groups.

Group 1 (SF6)

The mean age was 61.82±7.76 years (range 33-77 years) with male to female ratio of 1: 2.9 (17/66 males and 49/66 females). Mean duration of symptoms was 106.2 days (range 4-720 days) (Table 1). Optical coherence tomography based staging of macular holes was done with the distribution being 16.7%, 27.3%, 42.4% and 13.6% for stage 1,2,3 and 4 respectively.

Preoperative visual acuity in logMAR was 0.900 ± 0.383 which improved post macular hole surgery to 0.629 ± 0.375 (p=0.000). Careful measurement and documentation of preoperative and postoperative intraocular pressure by applanation tonometry revealed an increase of 1.99 mm of Hg (from

<table>
<thead>
<tr>
<th>Table 1: Patients- Demographic profile</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>Group 1(SF6), n=66</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>mean</td>
</tr>
<tr>
<td>range</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male, n (%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
</tr>
<tr>
<td>Laterality</td>
</tr>
<tr>
<td>Right eye , n (%)</td>
</tr>
<tr>
<td>Left eye, n (%)</td>
</tr>
<tr>
<td>Duration of symptoms (days)</td>
</tr>
<tr>
<td>mean</td>
</tr>
<tr>
<td>range</td>
</tr>
</tbody>
</table>

*chi square test       #mannwhitney u test
mean preoperative intraocular pressure of 14.12 ± 1.91 mm of Hg (as measured on last visit before surgery) to 16.11± 4.34 mm of Hg (maximum recording within 1 month post operatively). This rise in intraocular pressure was statistically significant (p=0.000). However incidence of glaucoma within 6 months of surgery was 6.1% (4/66 eyes). Preoperative examination of lens by slit lamp biomicroscopy revealed presence of clear lens in 9.1% (6/66 eyes), cataractous changes in 63.6% (42/66 eyes) and pseudophakic status in 27.3%(18/66 eyes). Out of these 16.7% (11/66) eyes had cataract removal with intraocular lens implantation were within 6 months of macular hole surgery. Surgical technique employed was sole discretion of the surgeon and 59.1% (39/66) of the eyes were operated using 20 gauge technique while 23 gauge was employed in 40.9% (27/66) of the eyes. 3 eyes had intraoperative retinal bleed near disc during internal limiting membrane peeling which stopped spontaneously.

Anatomical hole closure rate of 86.4% (57/66 eyes) was achieved after first surgery. Out of the 9 eyes with failed hole closure, 5 underwent resurgery. Additional internal limiting membrane (ILM) peeling and choice of gas in second surgery were left to surgeon’s discretion. 2/5 eyes were reinjected with SF6 out of which 1 eye achieved successful anatomical hole closure while 3/5 eyes received C3F8 as the tamponading agent and all 3 eyes had successful anatomical outcome but this closure rate of 80% (4/5 eyes) after resurgery did not translate in improvement in best corrected visual acuity (p=0.066) in these 5 eyes.

**Group 2 (C3F8)**

The mean age was 62.10± 7.64 years (range 29-79 years) with male to female ratio of 1: 2.6 . Mean duration of symptoms was 101.6 days (range 3- 720 days) (table 1). Optical coherence tomography based staging of macular holes showed increased prevalence of stage 3 holes in this group, 63.1% as compared to 42.4 % in group 1 but there was no statistically significant difference regarding stage wise distribution among the 2 groups (p=0.99). Mean preoperative visual acuity in logMAR was 1.03 ± 0.39 which improved post macular hole surgery to 0.61 ± 0.40 (p=0.000). An increase in mean intraocular pressure of 4.02 mm of Hg within 1 month of macular hole surgery was seen and this change was statistically significant.

Mean preoperative visual acuity in logMAR was 1.03 ± 0.39 which improved post macular hole surgery to 0.61 ± 0.40 (p=0.000). However incidence of glaucoma within 6 months of surgery was 9.0% (10/111 eyes). 26.1% (29/111) eyes were subsequently operated for visually significant cataract within 6 months of macular hole surgery. 20 gauge vitrectomy was performed in 47.7% (53/111) of the eyes while 52.3% (58/111) of the eyes were operated using 23 gauge vitrectomy system. 8 eyes had retinal touch during surgery.
### Table 2: Patients- Preoperative and intraoperative details

<table>
<thead>
<tr>
<th></th>
<th>Group 1, n=66</th>
<th>Group 2, n=111</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preoperative BCVA (logMAR)</strong></td>
<td></td>
<td></td>
<td>0.03*</td>
</tr>
<tr>
<td>Mean</td>
<td>0.90</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.2 – 1.5</td>
<td>0.2 – 1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Preoperative intraocular pressure (mm of Hg)</strong></td>
<td></td>
<td></td>
<td>0.78*</td>
</tr>
<tr>
<td>Mean</td>
<td>14.12</td>
<td>14.21</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>10 – 20</td>
<td>10 – 22</td>
<td></td>
</tr>
<tr>
<td><strong>Preoperative lens status</strong></td>
<td></td>
<td></td>
<td>0.68#</td>
</tr>
<tr>
<td>Clear, n (%)</td>
<td>7 (10.6%)</td>
<td>9 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>Cataract, n(%)</td>
<td>52 (78.8%)</td>
<td>86 (77.5%)</td>
<td></td>
</tr>
<tr>
<td>Pseudophakia, n (%)</td>
<td>7(10.6%)</td>
<td>16(14.4%)</td>
<td></td>
</tr>
<tr>
<td><strong>Preoperative macular hole staging</strong></td>
<td></td>
<td></td>
<td>0.06#</td>
</tr>
<tr>
<td>Stage 1, n (%)</td>
<td>11 (16.7%)</td>
<td>10 (9%)</td>
<td></td>
</tr>
<tr>
<td>Stage 2, n (%)</td>
<td>18(27.3%)</td>
<td>20 (18%)</td>
<td></td>
</tr>
<tr>
<td>Stage 3, n (%)</td>
<td>28(42.4%)</td>
<td>70 (63.1%)</td>
<td></td>
</tr>
<tr>
<td>Stage 4, n (%)</td>
<td>9(13.6%)</td>
<td>11 (9.9%)</td>
<td></td>
</tr>
<tr>
<td><strong>Gauge</strong></td>
<td></td>
<td></td>
<td>0.14#</td>
</tr>
<tr>
<td>20g, n (%)</td>
<td>39(59.1%)</td>
<td>53 (47.7%)</td>
<td></td>
</tr>
<tr>
<td>23g, n (%)</td>
<td>27(40.9%)</td>
<td>58 (52.3%)</td>
<td></td>
</tr>
<tr>
<td>Intraoperative complications, n (%)</td>
<td>3 (4.5%)</td>
<td>10 (9%)</td>
<td>0.20#</td>
</tr>
</tbody>
</table>

*Independent sample test  #chi square test

### Table 3: Post operative functional and anatomical outcomes ﾂ

<table>
<thead>
<tr>
<th></th>
<th>SF₆</th>
<th>C₃F₆</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre-operative</td>
<td>post-operative</td>
<td>P pre-operative</td>
</tr>
<tr>
<td>Anatomical hole closure, n(%)</td>
<td>0 (0%)</td>
<td>57 (86.4%)</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td><strong>BCVA (logMAR)</strong></td>
<td>0.90</td>
<td>0.62</td>
<td>&lt;0.05#</td>
</tr>
<tr>
<td>Lens status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear, n (%)</td>
<td>7 (10.6%)</td>
<td>6 (9.1%)</td>
<td>9(8.1%)</td>
</tr>
<tr>
<td>Cataract, n(%)</td>
<td>52(78.8%)</td>
<td>42(63.6%)</td>
<td>86(77.5%)</td>
</tr>
<tr>
<td>Pseudophakia, n(%)</td>
<td>7 (10.6%)</td>
<td>18(27.3%)</td>
<td>0.01*</td>
</tr>
<tr>
<td><strong>Intraocular pressure (mm of Hg)</strong></td>
<td>14.12</td>
<td>16.11</td>
<td>&lt;0.05#</td>
</tr>
<tr>
<td>Glaucoma, n(%)</td>
<td>0 (0%)</td>
<td>4(6.1%)</td>
<td>0.11*</td>
</tr>
</tbody>
</table>

* after first surgery  *chi square test  #wilcoxon signed rank test

marr whitney u test  *fisher exact t test
while 1 eye had peripheral retinal tear which was subsequently lasered intraoperatively. Intraoperative retinal bleed was seen in 1 eye which stopped spontaneously.

Anatomical hole closure rate of 86.5% (96/111 eyes) was achieved after first surgery. 15 eyes had failed hole closure out of which four underwent resurgery. 3/4 eyes were reinjected with SF6 while 1/4 eyes received C3F8 as the tamponading agent and all 4 eyes had unsuccessful anatomical outcome with persistent holes.

Comparative outcome analysis between group 1 and group 2

There was no difference in anatomical hole closure rates between group 1 and group 2, 86.4% and 86.5% respectively (p=0.982). There was an improvement in mean logMAR BCVA of 0.27 and 0.41 in group 1 and 2 i.e. higher in group 2 but the difference was not statistically significant (0.066). Rise in mean intraocular pressure of 1.99 mm Hg and 4.02 mm Hg were seen in group 1 and 2 (p<0.05 ). Incidence of glaucoma though higher in group 2 (9.0% versus 6.1%) was not statistically significant (p=0.482). 26.1% of eyes
in group 2 while 16.7% of eyes in group 1 underwent surgery for visually significant cataract within 6 months of macular hole surgery (p=0.146).

In case of 5 eyes which underwent resurgery in group 1, 80% (4/5) eyes had successful hole closure while none of the four eyes in group 2 met successful outcome.

Across the spectrum, 9 eyes underwent reinjections, among which SF6 was preferred on 5 occasions with hole closure rate of 20% (1/5 eyes) while 4 eyes received C3F8 with closure rate being much higher, that is 66% (3/4 eyes) but statistical significance could not be established because of small sample size of eyes which underwent resurgeries (Table 4).

**Table 4: Anatomical outcome after resurgery**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n=5)</th>
<th>Group 2 (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SF6*</td>
<td>C3F8*</td>
</tr>
<tr>
<td>Open#, n</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Closed#, n</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

*gas used in resurgery  
#status of macular hole after resurgery
DISCUSSION

Pathology in idiopathic macular hole has been believed to be the tangential and anteroposterior traction on the inner layers of macula. Vitrectomy, internal limiting membrane peeling and gas tamponade are integrated in one procedure to eliminate these pathologies. Vitrectomy is hypothesized to aid in macular hole closure by relieving the anteroposterior traction at the macula and create a space for an endotamponading agent. Various tamponading agents have been used ranging from room air, SF_6 gas, C_2F_6 gas, C_3F_8 gas, silicon oil, balanced salt solution, and perfluorocarbon liquids. Use of silicon oil instead of gases for faster visual rehabilitation has been compounded with lower success rate and the disadvantage of having its own set of complications with the need for resurgery for removal. Gas tamponade is believed to enhance macular hole closure by removal of tangential force by exerting floatation force at the macula, it also acts as a template for regeneration of inner retinal cells. ILM peeling is widely considered to facilitate macular hole closure by removing the tangential traction around the hole.

It has been a matter of debate regarding the duration of tamponade required for closure of macular hole. Various studies have been conducted with different endotamponading agents mentioned above with considerably good results. In the current study, no significant difference was observed regarding the anatomical hole closure rates between SF6 and C3F8 group. Similar outcome had been deduced by Kim et. al., though in a smaller cohort of patients. In a study by Jackson et. al. various gas mixtures including air, SF6, C3F8, and C2F6 were used as endotamponading agents with no significantly different macular hole closure rates. Spaide in his study advocated that in case of early macular holes only vitrectomy without gas injection is enough for macular hole closure while Mori K et. al. in their case series involving stage 2 macular holes only, found 100% closure rate with intravitreal gas injection without vitrectomy in patients with preoperative visual acuity better than 20/40 and 90% closure rate in patients with hole diameter less than 200 microns. Posturing has been advocated since the advent of macular hole surgery as an important determinant for macular hole closure but Rehman R8 and Nadal J10 got excellent anatomical closure rates without any posturing in their patients. Postoperative positioning was however not studied as a determining factor in the current study.

In the current study there was significant improvement in visual acuity postoperatively in both the groups and though more improvement was noted in C3F8 group, the difference between the 2 groups was not significant. Similar results were obtained in the study by Kim et. al. while Usui et.
al. compared visual outcomes among SF6 and air as the endotamponade agent and found no difference among the 2 groups. Various factors such as symptom duration of less than 1 year, earlier macular hole stage, intraoperative internal limiting membrane peeling yielded the best visual acuity after successful surgical repair in various studies but were not analysed in the current study.

As far as adverse events after the surgery were concerned, rise in intraocular pressure, incidence of glaucoma and visually significant cataract necessitating surgery were higher with C3F8 gas as the tamponade agent in the current study but the difference between the 2 groups was not significant. No difference was observed in development of vision-impairing cataract in phakic eyes by Kim et. al., but the myopic shift was greater in the C3F8 group. In Jackson’s study, high incidence of cataract surgeries (63%) were reported within 1 year of macular hole surgeries but they used at least 4 different types of gas mixtures as tamponade. Thompson in his study made some interesting observations as to the fact that progression of nuclear sclerosis was significantly more in older age group while there was negligible progression in posterior subcapsular cataract in any age group, and progression of cataract was much more with intraocular gas use than cases in which vitrectomy alone was performed. Other adverse events like pupillary capture were found to be more in C2F6 group than SF6 group but no such events occurred in the current study. As far as reinjection of gases was concerned, C3F8 performed better than SF6 in achieving successful anatomical hole closure, more so in group 1 than group 2 even though it did not translate in achieving corresponding visual success.

Definitely a study with large sample of reoperated cases in failed first macular hole surgery is required to attach any significance to the above mentioned observations. And researchers have been doing extensive work on this class of patients with varied recommendations. Rao and Johnson in their studies achieved 89% and 74% success rate with outpatient fluid gas exchange in cases with failed first surgeries while Imai recommended additional intravitreal gas injection within 2 weeks of initial surgery. Ohana and Ikuno went a step further and advocated use of focal laser to foveal pigment epithelium along with fluid gas exchange in these patients.

Limitations of the current study include its retrospective design (i.e., the lack of randomization), the limited postsurgical follow-up, longer follow-up in patients receiving the C3F8 gas and smaller sample of patients undergoing resurgeries.

Thus SF gas is comparative in its effect to C3F8 and has a significant advantage of lesser duration of tamponade which means lesser debility especially
in one eyed patients, faster return to normal daily routine and sooner air travel. Probably the only advantage \( C_3F_8 \) gains over SF6 is the success rate in resurgeries but to prove credibility of the point, a comparative study with a large sample size is required.

REFERENCES


Inverted ILM Flap Technique for Closure of Large Macular Holes

Dr. Unnikrishnan Nair R, Dr. Manoj S, Dr. Nair Ramachandran K G

The surgical management of idiopathic macular holes has been revolutionized by the introduction of Vitrectomy as the primary modality of treatment by Kelly and Wendel in 1991. ILM peeling as an adjuvant to vitrectomy ushered in both debate and enthusiasm as to its efficacy. The success rate of macular hole surgery with ILM peeling has been reported to be 92 to 98%. ILM peeling as an integral step has been found to be significant by studies by Lois et. al. and the FILMS study group that have concluded that there is no statistically significant difference in distance visual acuity between the groups that have ILM peeling or those without, but the ILM peeled eyes had significantly better closure rates and less re-operation rates.

The configurations of closure after macular hole surgery has been termed as Type 1 and Type 2 closure. In Type 1 closure the ends of the neuro-sensory retina meet and are also in approximation to the RPE. In Type 2 closure the ends of the neuro-sensory retina are in close proximity to the RPE but there is a persistent defect between the edges. The absence of retinal elements in the centre of the fovea usually results in a visual acuity less than expected compared to a case where there has been type 1 closure.

Type 2 closure has also been termed as Flat Open configuration where the hole is flat with exposed or “open” RPE. Various adjuvants have been used to ensure closure of the central retinal elements better and hence Type 1 closure. These include the use of TGF-β2, autologous serum, fibrin glue, gelatin pugs, bovine thrombin etc. The results have been comparable to the closure rates as compared to vitrectomy with or without ILM peeling, with no additional benefit.

The significance of these modifications comes to the fore as management of larger and larger holes are being attempted. Most studies include the 400 microns as the definition of a large hole. A newer modification for management of large macular holes has been the introduction of the inverted ILM flap technique. This technique involves performing the ILM peeling to the edge of the hole and repositing the ILM remnants in the base of the hole. The main visible outcome seen would be the prevention of flat open configuration of closure and the benefits associated with this. This study attempts to study the benefits and safety of the inverted ILM flap techniques for extra large holes greater than 800 microns in diameter.
MATERIALS AND METHODS

This was a prospective study of patients who had undergone macular hole surgery for holes exceeding the diameter of 800 microns at Chaithanya Eye Hospital and Research Institute between November 2010 and March 2013. Forty patients with larger macular holes with a greatest linear diameter of more than 800 microns were included in this non randomized interventional study.

Data collected from the patient charts included patient age and gender, lens status prior to surgery, preoperative visual acuity, other retinal and ocular diseases, stage of the macular hole, postoperative visual acuity, hole closure status after surgery, type of hole closure, whether or not cataract surgery was done during vitrectomy or later during follow-up, list of any complications and final follow-up duration in months. This was followed by a detailed evaluation and assessment with the SD OCT (Heidelberg Spectral is).

Twenty patients underwent traditional 23G Vitrectomy with ILM peeling and injection of SF6 (Group 1). The other group of twenty patients underwent 23G Vitrectomy followed by the inverted ILM flap technique and injection of SF6 (Group 2).

Inverted ILM flap Technique

The inverted ILM flap technique involves staining the ILM with Brilliant Blue G (BBG). This is followed by peeling the ILM to the edge of the hole and trimming it. The ILM is then reposited inside the hole and subsequently a fluid air exchange (FAE) is done. The air is then replaced with 16% SF6. The patient is advised a prone position for 5-7 days.

Significant cataract was defined as more than NS2, any PSCC or cortical cataract involving the visual axis. Epiretinal membrane (ERM) peeling was performed whenever required. Patients who developed any significant cataract in this period, which was responsible for the visual deficit, underwent cataract surgery as and when required. All Snellen’s visual acuities were converted to logMAR for purposes of analysis.

RESULTS

Baseline Characteristics and procedural details

40 eyes of 38 patients were included in the study. The mean age was 59.7 years (range 51–72). There were 16 males (42.1%) and 22 females (57.9%). 29 eyes (72.5%) had stage 3 macular holes, and 11 eyes (27.5%) had stage 4 macular hole. The mean duration of macular hole evaluated from history was 15.58 months (range 8 to 28 months).The mean basal diameter of
macular hole was 1091.01 microns (range 874 microns to 1660 microns). OCT features included CME and SRF in all eyes (100%), incompletely detached posterior hyaloid in 22 eyes, completely detached posterior hyaloid in 11 eyes and no posterior hyaloid detachment in 7 eyes. At baseline, 26 eyes (65%) were phakic, and 14 eyes (35%) were pseudophakic. All eyes underwent 23G vitrectomy while 13 eyes (32.5%) underwent 23G vitrectomy combined with Phacoemulsification and IOL implantation. The mean follow-up was 6.2 months with a minimum follow-up of 4 months. Out of the remaining 13 phakic eyes, 8 eyes developed significant cataract in the early post operative period and needed early cataract surgery with IOL implantation within 3 months of follow up.

**Closure of the macular hole**

16 of the 20 Group 1 Eyes closed after the procedure. Of these 13 eyes had type 1 closure and three eyes had type 2 or flat open configuration. Of the remaining four eyes, one underwent Re-peeling with intra-ocular gas at six weeks and subsequently closed with type 1 closure. This makes the final hole closure rate 85%.

All twenty of the group two eyes closed with a type 1 closure at the end of 6 weeks. At 3 months there was an apparent loss of retinal elements at the outer retinal layers in one eye with conversion to a flat open type configuration. The hole closure rate was 100% with a flat open configuration in only in one eye.

**Functional Results**

The mean preoperative visual acuity was 1.22 logMAR (6/120 Snellen equivalent)- range 6/24 to CF1M). The mean postoperative vision was 0.72 logMAR (6/36 Snellen equivalent)- range 6/18 to 6/60. Out of the 40 eyes
30 eyes (75%) showed improvement in vision while in 7 eyes (17.5%) the vision remained status quo and in 3 eyes (12.5%) the vision dropped from baseline. Group 1 eyes the post operative Va of the group was 0.78. In the Group 2 eyes the preoperative Va was 0.62.

When comparing the type of hole closure with visual outcome it was found that 24/33 eyes (72.7%) with type 1 closure had vision improvement while the remaining 7 had stabilization, 2/4 eyes (50%) with type 2 closure had vision improvement.

In 27.7 % eyes with type 1 closure and 50% of type 2 closure there was no visual gain. Both the 3 eyes where the hole remained open after surgery (100%) had poor visual outcome.

**Post Operative OCT**

In the Early post operative period there was appearance of a thin layer superficially over the hole (ILM flap) with evidence of sliding of retinal elements. Later evaluation showed increase in thickness at tissues at the base of the hole. Often the demarcations corresponding to the original base of hole were visible.

**DISCUSSION**

The Gold standard surgical management of Macular Holes is now widely considered to be Vitrectomy with ILM peeling. However in larger macular holes of longer duration the success rate of surgery has not been as encouraging as in holes smaller than 400 microns. Various modifications in the technique of ILM peeling has been considered including mechanical closure of hole, use of adjuants, removal of SRF at base of the hole and fluidic separation of the ILM. The inverted ILM flap technique id one such newer modification described by Michalewska et. al. in 2010 for larger macular holes. This technique involves peeling ILM around the macular hole and retaining a rim of ILM around the edge of the hole and reposting it in the macular hole before fluid air exchange. This study applies this technique to
holes larger than 800 microns. The present study found better hole closure rates with type 1 closure in the group where the Inverted ILM flap technique was used. The need for re-surgery was also not seen on this group. In the group where routine ILM peels were done, there was a final non closure rate of nearly 15%.

However even in the eyes with closure 17.6% (3/17) had a flat open configuration. That is the type 1 closure was limited to 70% (14/20) which means that 30% eyes has exposed RPE even after the procedure. There was no statistically significant difference between post-operative final visual acuities however the post-operative visual acuity in the group 2 eyes was apparently better. But generally it was seen that eyes that has type 1 closure had better rates of visual gain than eye with type 2 closures. The long term implications of having an exposed RPE is to be seen and there is a high likelihood that such an exposure to the vitreous current is likely to cause RPE damage in the long run.

The principle of the inverted flap techniques is to induce glial cell proliferation, resulting in the macular hole filling with proliferating cells that enhance closure. In a healthy retina, neurons are surrounded by Müller cells. If the retina is detached or otherwise damaged, macrophage-like cells from the vitreous\(^{10}\) may infiltrate the retina. These cells may activate Müller cells via tissue necrosis growth factor-α\(^{11}\) inducing gliosis.\(^{12,13}\) Moreover, the peeled-off ILM itself contains Müller cell fragments; therefore, ILM peeling alone can induce gliosis. Thus, if a segment of peeled-off ILM is left attached, it may provoke gliosis both inside the retina and on the surface of the ILM. The ILM also may be a scaffold for tissue proliferation. This hypothesis is based on histo-pathological findings suggesting that cells always need a basement membrane to proliferate.

The other mechanism may be that the ILM reposited segregates the base of the hole from the thin film of fluid that is present in the vitreous cavity after intraocular tamponade. This may hinder the migration or sliding of the adjacent retinal layers on to the base of the hole. A base devoid of the fluid cuff may allow for adhesion of the retinal layer that have slid in after removal of tangential traction. This may also be the hypothetical mechanism of action while using adjuants like thrombin plugs, fibrin glue and autologous serum.

In the study by Michalewska, macularhole closure was observed in 88% of patients in group 1 and in 98% of patients in group 2. A flat-hole roof with bare retinal pigment epithelium (flat-open) was observed in 19% of patients in group 1 and 2% of patients in group 2. In our study the hole closure rates were 85% and 100% respectively in each group and the flat open configuration was seen in 17.6% of group 1 and 5% of group 2.
While there is a concern that leaving a frill of ILM at the edge of the hole goes against traditional knowledge that the ILM must be peeled across the hole, the study by Michalewska and ours prove that the hole closure is unaffected by this and moreover show better results. This may be because a large peel around the hole releases the tangential traction sufficiently enough to allow the retinal elements to slide in toward the centre. The other concern is that the ILM repositioned into the hole is not neural tissue and hence it is only an illusionary closure and not a true type 1 closure where there are actually neuro-sensory elements in the macular centre.

However both studies demonstrate a significant increase in visual acuity and functional improvement given the fact these were done for large chronic holes. Here we believe that the ILM may act as scaffolding for sliding of the neural elements besides glial proliferation.

The learning curve is very fast with the only single drawback being a careless approach may lead to ripping off the ILM rather than retaining a frill. Sometimes even in the most careful hands the ILM remnants may avulse off during FAE.

Hence in case of extra large macular holes as in this study, rather than techniques employing mechanical closure and FAE at base of hole (trauma to the retinal and RPE elements), use of autologous serum, fibrin plugs, tissue adhesives (introduction of extraneous material) or very large peels (micro-trauma to RNFL), the inverted ILM flap technique just becomes a surgical extension of one’s own ILM peeling technique and of the safest options available to close such holes.

This technique has few complications and a fast learning curve for a surgeon already well versed in ILM peeling. This technique is a innovative addition to one’s surgical armamentarium and will give the retinal surgeon great confidence in tackling very large macular holes.

REFERENCES


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**Predicting Outer Layer Anatomical Restoration after Macular Hole Surgery Using Spectral Domain OCT**

**Dr. Rameez Najamul Hussain**, Dr. Giridhar A, Dr. Mahesh G, Dr. Santosh Singh Patel

Once OCT became available in outpatient clinics, attempts were made to assess hole measurements and predict surgical success. But outer layer restoration could not be predicted with the time domain OCT. This study was designed to predict the outer layer anatomical restoration after macular hole surgery. As studies have shown that External limiting layer restoration
is associated with positive visual outcome, predicting its restoration will guide the surgeon to prognosticate the surgery.

**Purpose**

To study the preoperative micro morphological indices (seven indices) in full thickness macular hole measured using spectral domain OCT (SD-OCT) namely; Macular Hole Height (MHH), Base Diameter (BD), Minimum Linear Diameter (MLD), Macular Hole Inner Opening (MHIO), Tractional Hole Index (THI), Macular Hole Index (MHI), Hole Form Factor (HFF) and correlate with postoperative anatomical outcome (Restoration of outer layer anatomical restoration-ultra structural morphology of External Limiting Membrane (ELM) and Inner segment/Outer segment (IS/OS) junction and macular hole type 1 closure).

**MATERIALS AND METHODS**

A prospective case series study of 69 eyes of 69 patients who was diagnosed with full thickness macular hole. All eyes underwent a comprehensive clinical evaluation including presenting best corrected visual acuity, anterior segment bio-microscopic examination, direct and indirect fundus examination.

SD-OCT imaging was done for all eyes (Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany) in high definition dense scan mode; 20°x20° area with 9 raster B-scans, each composed of 768 A-scans and resulting from an average of 9 frames. The scanning machine has an SD-OCT technology with a confocal scanning laser ophthalmoscope (cSLO). At the baseline examination, each SD-OCT B-scan was registered and saved as a reference image.

**Macular hole indices were calculated from images (Figure 1)**

1. Macular Hole Height (MHH) - vertical length between the retinal pigment epithelial layer and the highest portion of the macular hole (Figure 1. measurement e).
2. Base Diameter (BD) - distance measured at the level of retinal pigment epithelium (Figure 1. measurement a).
3. Minimum Linear Diameter (MLD) - minimal extent of the hole (Figure 1. measurement b).
4. Macular Hole Inner Opening (MHIO) - distance between the innermost layer in the macular defect (Figure 1. measurement f).
5. Tractional Hole Index (THI) - e / b (b=MLD)
6. Macular Hole Index (MHI) - e / a (b= MLD, a= BD)
7. Hole Form Factor (HFF)\[7 - \frac{c + d}{a}\] (c= left arm length, d= right arm length, a= base diameter (Figure 1).

All cases underwent uncomplicated 23G vitrectomy (triamcinolone assisted) with internal limiting membrane (ILM) peeling. After peripheral truncation of the vitreous base, brilliant blue dye was used to stain the ILM. Once ILM peeling was done across the hole and 360° around the edge of the macular hole. Fluid air exchange was done and gas tamponade by C\(_3\)F\(_8\) or SF\(_6\) was performed. All cases were done by a single experienced Vitreo-retinal surgeon. Post operative prone positioning was advised for all cases for 4 days.

SD-OCT images were obtained in the study eyes in follow up mode after 3 months. In the follow up scanning, the eye-tracking system software guided the SD-OCT laser to scan the same location again which was saved in the base line examination. In this way, accurate follow up scan images were obtained and evaluated.

Anatomical closure, ELM restoration and IS/OS junction layer integrity were studied and correlated with pre-operative indices.

**Statistical analysis**
Statistical analysis was done with IBM SPSS Windows V.16.0. Independent two sample T test. Mann Whitney test and the association between categorical variables were done with Chi-square test and Fisher exact test.

**RESULTS**
18(26.8%) were males and 51(73.2%) were females. The mean age was 63.58 ±6.24 years.

Macular hole closure or anatomical success was seen in 61 patients (88.4%) and was not in remaining 8 patients (11.6%). Among the 7 pre-operative macular hole indices, 2 were found to have significant association with anatomical success. Minimum Linear Diameter (MLD) (p=0.047) and Base Diameter (BD) (p=0.041) showed statistical significance. (Table 2)

The ELM layer was formed in 59 patients (85.5%) and not formed in 10
patients (14.5%). The IS/OS layer was not formed in 27 patients (39.13%) and formed in 42 patients (60.86%).

External limiting membrane (ELM) restoration had significant correlation with macular hole index (MHI). It had an inverse correlation with ELM restoration. Restoration of Inner segment-outer segment junction (IS OS) didn’t show any significant correlation with the indices.

**DISCUSSION**

The first study to use OCT to analyse macular holes preoperatively was published by Ip et. al.\(^8\) in 2002. It has been named the MLD or the ‘maximum minimum linear dimension’ taking into account the need for accurate selection of an OCT image that represents the true extent of the hole. Significant correlations between MLD and visual outcomes after macular hole surgery have been described.\(^10\)

The logistic regression analysis done by Laura et. al.\(^6\) in a study indicated that the basic ophthalmic parameters base diameter, macular hole inner opening and MLD are associated with both anatomical and visual success, and exhibit good discrimination with respect to either outcome measure.
In our study, ELM restoration had significant correlation with Macular hole index. This means that outer layer restoration can be predicted with macular hole index and thereby we can indirectly predict the visual outcome. Post operative IS OS layer integrity had no significant correlation with any of the pre operative measurements. This may be due to the timing e.g. post-operative OCT. Studies have shown that IS OS later restoration is seen more than 3 months time.

Minimum Linear Diameter (MLD) and Base Diameter (BD) were predictive of anatomical closure. A simple measurement of the distance at the level of retinal pigment epithelium and minimal extent of the hole can predict the chances of anatomical closure of the macular hole. In this study, anatomical success was achieved in 61 eyes (88.4%) with base diameter less than 832.9±718.9 microns and MLD less than 362.9 ± 221.3 microns. This is comparable to the studies done by Ip et. al.⁸

This study correlates all the validated pre operative macular hole indices with anatomical outcome and thereby guiding the vitreo retinal surgeons to choose and give prognostic value for the surgery.

**CONCLUSION**

Pre-operative micro morphological indices in full thickness macular hole measured using spectral domain OCT (SD-OCT) can predict the anatomical outcome of macular hole surgery. Minimum Linear Diameter (MLD) and Base Diameter (BD) were predictive of anatomical closure while Macular Hole Index (MHI) predicted outer layer restoration (ELM formation).

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**Clinical Outcomes of Double Membrane Peeling for Vitreoretinal Interface Disorders**

**Dr. Kshitiz Kumar, Dr. Atul Dhawan, Dr. Amar Agarwal, Dr. Athiya Agarwal**

Spectral domain OCT has deeply enhanced the understanding of vitreoretinal interface (VRI) and its disorders. The various VRI associated disorders are posterior vitreous detachment, vitreomacular traction syndrome (VMTS), idiopathic epiretinal membrane (ERM) and macular pseudoholes (MPH), lamellar (LMH) and full thickness macular holes (FTMH).¹,² ERM results from proliferative changes at the VRI, either secondary to other ocular conditions or it can idiopathic in nature. Partial
or complete PVD is found in 80% to 95% of eyes with idiopathic ERM, and the latter was suggested to be secondary to vitreous schisis and vitreous remnants on the retina promoting subsequent epiretinal fibrocellular proliferation. ERM is commonly associated with various subtypes of VRI disorders. Koizumi et al. performed a three-dimensional evaluation of the VRI in VMT syndrome using SD-OCT. They found that most of the eyes with VMT syndrome had concurrent epiretinal membranes. ERM has been shown to coexist in most cases of macular pseudohole (MPH) and lamellar macular hole (LMH). Michalewski et al. used SD-OCT to demonstrate that MPH may progress to LMH, and because it is an advanced stage of the same non–full-thickness macular disorder, progression of ERM may be the cause of both MPH and LMH. Chen et al. hypothesized that both entities may be different manifestations of the same disease.

Role of ILM peeling in the successful management of idiopathic full thickness macular hole is already well established. ILM removal to prevent the recurrence of ERM, which is roughly 10-16.3%, has been advocated recently. Beneficial effect of ILM peeling with intraocular gas tamponade was suggested for pseudolamellar hole (PLH) and vitreomacular traction (VMT) type ERM cases. ERM removal alone did not improve vision in PLH cases with highest recurrence rate in VMT type of ERM. However, concerns regarding microtrauma to sensory retina and toxicity by the staining dye have made ILM removal during surgery controversial.

In the present study, we aimed to evaluate the clinical outcomes of double membrane (ERM and ILM) peeling with or without combined phacovitrectomy and intraocular gas tamponade in four types of VRI disorders: ERM, Pseudoholes, Lamellar Holes and VMTS.

**MATERIALS AND METHODS**

**Study design**

This study followed the ethical standards established in the Declaration of Helsinki and was approved by the institutional review board of Dr. Agarwal’s Eye Hospital. We retrospectively reviewed the medical records of consecutive patients who underwent vitrectomy for idiopathic vitreoretinal interface (VRI) abnormalities, except full-thickness macular hole, between November 2012 and November 2013.

Patients were included in the study if they were followed-up for at least 6 months post surgery. Patients with previous history of vitrectomy, other accompanying macular disorders, retinal detachment, retinal vascular disease, or diabetic retinopathy were excluded from this study. Initially,
115 eyes of 104 patients who underwent vitrectomy for VRI associated pathologies during the study period were reviewed. Finally, 72 eyes of 72 patients who met the criteria were included for data analysis.

**Surgical technique**

All procedures were performed by a single surgeon (AA) at Dr. Agarwal’s Retina Foundation, Chennai, India. A standard 23-gauge 3-port pars plana vitrectomy was performed. Phacoemulsification and intraocular lens implantation through a 3.0 mm temporal clear corneal incision was performed before vitrectomy, if indicated.

After core vitrectomy, triamcinolone acetonide (TA) was injected into the vitreous cavity to determine if posterior vitreous detachment had occurred. For cases without posterior vitreous detachment, vitreous cutter switched to the vacuum mode was used to induce posterior vitreous detachment. The residual TA particles would stain the vitreoretinal interface along with ERM on the macula. This ensured complete identification and peeling of ERM with 23G ILM forceps (Grieshaber Revolution DSP). After ERM removal, the ILM was stained using Brilliant Blue G (BBG), 0.2 mL with a concentration of 0.25 mg/mL, 0.025% (Brilliant Peel; Geuder, Heidelberg, Germany) on the macula under air conditions for 2 minutes. ILM was peeled easily and safely for about two disc diameter size in the same fashion. Peripheral retinal indentation was done to check for any retinal breaks. SF6 (20%) intraocular gas tamponade was performed depending upon the discretion of the surgeon.

**Data collection**

Best-corrected Snellens visual acuity (BCVA), Optical coherence tomography data using Cirrus HD-OCT (Carl Zeiss Meditec Inc., Dublin, California) were collected at baseline and postoperative 1, 3 and 6 months, apart from routine slit-lamp and fundus examination.

**Classification of Vitreo-retinal Interface Disorders**

The macula was scanned in the horizontal and vertical meridians using a standard, linear, cross hair pattern with a scan length of 6 mm and was centered through the fovea as determined by simultaneous evaluation of the red-free image on the computer monitor of the OCT scanner. All preoperative OCT scans were classified into VMTS, ERM, MPH and LMH, by an independent masked observer (KK) as followed: Vitreomacular traction (VMT) syndrome was characterized by partial PVD with residual strong posterior vitreomacular adhesions, which can be focal foveolar adhesion and broad macular adhesion (where the maximum diameter of vitreomacular attachment is more than 1500μm). Epiretinal membrane
(ERM) with diffuse / cystoid edema was defined as a hyper reflective layer on the retina with variable adherence. Macular Pseudohole (MPH) was a hole in an epiretinal membrane (ERM) with steepened foveal pit but without any neurosensory defects in the retina. Lamellar Macular Hole (LMH) was defined as splitting of the inner and outer foveal layers, the absence of a full thickness defect, and the presence of irregular foveal contour. Example of these 4 types are shown in Fig.

Central foveal thickness (CFT) was calculated by one of the experienced examiners (KK) by manually averaging the foveal thickness of the horizontal and vertical images using the calliper tool built into the OCT software.

Statistical Analysis

All parameters used in the study were expressed as the mean ± standard deviation (SD). For calculation of the mean and subsequent statistical comparisons, Snellen’s units of BCVA was transformed to the logarithm of the minimum angle of resolution (logMAR) units.

Nonparametric Wilcoxon signed rank test was used to compare pre- and postoperative changes in BCVA and CFT within a single VRI disorder. Kruskal–Wallis test was used to compare age, BCVA, CFT among the types. Chi-square tests was used to compare gender among different morphological types. The correlation between BCVA and CFT was calculated by Pearson’s correlation tests. Subgroup analysis was performed using the Mann–Whitney U test to compare BCVA and CFT change in patients who underwent simultaneous phacoemulsification with IOL implantation versus those who did not undergo combined surgical procedure and those who received intraocular gas tamponade versus those who did not receive SF6 injection. p-values of < 0.05 were considered significant for all analyses.

RESULTS

Demographics and Preoperative characteristics

Seventy two eyes of 72 patients were included in this study. Male to female ratio was 1:1 with mean age at presentation being 64.90±6.9 years (range: 48-84). 35 (48.61%) eyes were pseudophakic at baseline. Four types of VRI disorders were, globally adherent ERM in 61.11% (44/72) eyes followed by VMTS in 23.61% eyes(17 /72); MPH and LMH were present in 9.7% (7 /72) and 5.5% (4/72) eyes respectively. Mean preoperative BCVA was 0.58± 0.14 logMAR units. Mean preoperative CFT was 409.17±122.31µm. There was no correlation between preoperative BCVA and CFT (r=0.136, p=0.2546). There was no significant difference in age, gender, and preoperative BCVA among groups. Preoperative CFT was significantly less in LMH group compared to other three types of VRI abnormalities (Kruskal–Wallis test, p=0.0031). (Table 1).
Postoperative Data

Mean postoperative BCVA was 0.27±0.16 logMAR units at 6 months, which significantly improved compared to preoperative BCVA (Wilcoxon signed rank test; p= 0.001). Mean postoperative CFT was 277.28±0.16 µm, which significantly reduced from the preoperative CFT (Wilcoxon signed rank test; p < 0.0001). There was no significant correlation between postoperative BCVA and CFT (r=-0.0599, p=0.6172). Combined phacoemulsification and intraocular lens implantation (PE/IOL) with pars plana vitrectomy was done in 35 eyes. Intraocular gas (SF6) tamponade was used in 58.33% (42/72) eyes.

Postoperative outcomes in VRI types

Table 1

<table>
<thead>
<tr>
<th>Type</th>
<th>ERM</th>
<th>VMTS</th>
<th>Pseudohole (MPH)</th>
<th>Lamellar Hole (LMH)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>No. of Eyes (72)</td>
<td>44</td>
<td>17</td>
<td>7</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>23:21</td>
<td>7:9</td>
<td>4:3</td>
<td>1:3</td>
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<td>Age (Mean ± SD), years</td>
<td>64.68±6.68</td>
<td>66±8.02</td>
<td>66.71±5.08</td>
<td>59.25±6.39</td>
<td>0.2079</td>
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<tr>
<td>Preoperative BCVA (Mean ± SD), logMAR</td>
<td>0.65±0.15</td>
<td>0.54±0.10</td>
<td>0.58±0.10</td>
<td>0.37±0.15</td>
<td>0.3224</td>
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<tr>
<td>Preoperative CFT (Mean ± SD), µm</td>
<td>436.72±98.92</td>
<td>426.23±129.46</td>
<td>359.57±81.07</td>
<td>142.75±20.43</td>
<td>0.0031</td>
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Table 2

<table>
<thead>
<tr>
<th>Post - operative BCVA (Mean ± SD), logMAR units</th>
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<td>3 months</td>
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<tr>
<td>ERM</td>
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<td>Lamellar Hole</td>
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<tr>
<td>p-value</td>
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<tr>
<td>p-value†</td>
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*Compared to baseline, Wilcoxon’s signed rank test; †Comparison between the groups at each time point, Kruskal-Wallis test.
There was significant visual improvement in all except for LMH variety (ERM type, p=0.0029; VMTS type, p=0.0281; MPH type, p=0.05; and LMH type, p=0.7926) and among the types, gain in visual improvement was similar (Kruskal-Wallis test; p=0.1014) at 6 months. (Table 2) Also mean postoperative CFT in lamellar macular hole type (129.25±12.28) was significantly different from other 3 types of VRI disorders (Kruskal-Wallis test; p=0.0042) (Table 3).

Postoperative outcomes in Phacoemulsification (PE/IOL) vs Pseudophakic group.

There was significant improvement in vision along with CFT reduction in both groups, postoperatively, at 6 months follow up visit (Wilcoxon signed rank test; p<0.05). Comparing eyes that had simultaneous PE/IOL versus pseudophakic group, respective post–op mean BCVA was 0.270±0.169 logMAR versus 0.277±0.162 logMAR units (Mann–Whitney U test; p=0.378), and post–op mean CFT was 260.40 ± 76.31 µm versus 288.42 ± 68.83 µm (Mann–Whitney U test; p=0.069) (Table 4).

Postoperative outcomes in Intraocular SF6 vs no Tamponade group

Improvement in vision with CFT reduction was significant in both groups at 6 months postoperatively (Wilcoxon signed rank test; p<0.05). Comparing eyes that had intraocular SF6 tamponade versus those without intraocular tamponade, respective mean post-op BCVA was 0.28±0.16 logMAR versus 0.25±0.16 logMAR units (Mann–Whitney U test; p=0.166), and the mean CFT was 266.81±76.62 µm versus 285.35±68.44 µm (Mann–Whitney U test; p=0.293) (Table 5).

DISCUSSION

SD-OCT has immensely helped in understanding and classifying various vitreoretinal interface (VRI) disorders and more intelligent information-based surgical decision making. This was the first study to group together various interface disorders of the macula, and find out surgical outcome following pars palna vitrectomy. FTMH was excluded from this study as it has already been studied extensively and the surgical procedure for it doesn’t confirm to double membrane (ERM & ILM) removal routinely.

On SD–OCT evaluation, apart from identifying Epiretinal membrane (ERM) as an individual entity, the latter was found to be associated with all VRI types. There is significant overlap between VMT and idiopathic ERM, and all 17 eyes in this series with VMT had concurrent ERM. Chang et. al. proposed mechanism of ERM proliferation in VMT syndrome. After partial posterior vitreous detachment, small splits within the ILM may form which allow glial cells to gain access to the superficial retina which acts as a scaffold.
### Table 3

<table>
<thead>
<tr>
<th></th>
<th>3 months</th>
<th>6 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERM</td>
<td>324.20 ± 73.34</td>
<td>291.54 ± 68.63</td>
</tr>
<tr>
<td>p – value*</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>VMTS</td>
<td>319±90.94</td>
<td>267.82 ± 61.19</td>
</tr>
<tr>
<td>p – value*</td>
<td>0.0004</td>
<td>0.0003</td>
</tr>
<tr>
<td>Pseudohole</td>
<td>313.85±71.57</td>
<td>261.71±61.93</td>
</tr>
<tr>
<td>p – value*</td>
<td>0.0276</td>
<td>0.2846</td>
</tr>
<tr>
<td>Lamellar Hole</td>
<td>136.50 ± 15.80</td>
<td>129.25±12.28</td>
</tr>
<tr>
<td>p – value*</td>
<td>0.3208</td>
<td>0.0925</td>
</tr>
<tr>
<td>p – value†</td>
<td>0.0042</td>
<td>0.0092</td>
</tr>
</tbody>
</table>

*Compared to baseline, Wilcoxon’s signed rank test; †Comparison between the groups at each time point, Kruskal-Wallis test.

### Table 4

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-Op BCVA (logMAR units)</th>
<th>Post-Op BCVA (logMAR units)</th>
<th>Pre-Op CFT(µm)</th>
<th>Post-Op CFT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE/IOL (35)</td>
<td>0.613 ± 0.125</td>
<td>0.270±0.169</td>
<td>377.05±126.32</td>
<td>260.405±76.31</td>
</tr>
<tr>
<td>p-value*</td>
<td>0.04</td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Pseudophakic (37)</td>
<td>0.611 ± 0.156</td>
<td>0.277±0.162</td>
<td>455.68±109.79</td>
<td>288.42±68.83</td>
</tr>
<tr>
<td>p-value*</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>p-value†</td>
<td>&lt;0.001</td>
<td>0.378</td>
<td>0.014</td>
<td>0.069</td>
</tr>
</tbody>
</table>

*Compared to baseline, Wilcoxon’s signed rank test; †Comparison between the two groups at each time point, Mann-Whitney’s U-test.

### Table 5

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-Op BCVA (logMAR units)</th>
<th>Post-Op BCVA (logMAR units)</th>
<th>Pre-Op CFT(µm)</th>
<th>Post-Op CFT (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With SF₆ Tamponade (42)</td>
<td>0.647 ± 0.131</td>
<td>0.286±0.166</td>
<td>415.31±144.87</td>
<td>266.81 ± 76.62</td>
</tr>
<tr>
<td>p – value*</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without Tamponade (30)</td>
<td>0.571 ± 0.148</td>
<td>0.253±0.164</td>
<td>402.71±77.89</td>
<td>285.35 ± 68.44</td>
</tr>
<tr>
<td>p – value*</td>
<td>0.009</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>p – value†</td>
<td>0.293</td>
<td>&lt;0.001</td>
<td>0.332</td>
<td>0.610</td>
</tr>
</tbody>
</table>

*Compared to baseline, Wilcoxon’s signed rank test; †Comparison between the two groups at each time point, Mann-Whitney’s U-test.
for ERM proliferation. These cells may also proliferate onto the detached hyaloid face, strongly anchoring the vitreous to the macula. In this study there were 4 cases of lamellar macular hole (LMH). Epiretinal membranes appear to have a role in the pathogenesis of LMH, and the former is found to be responsible for increase in diameter of LMH. ERM contraction present in 67-100% LMH cases depending on the type of OCT employed for detection may account for its progression. Macular Pseudohole (MPH) being an advanced stage of non-full-thickness macular disorder (NFMH), also results from progressive contraction of epiretinal membranes (ERM). ERM coexisted in 100% cases of NFMH which comprises of both MPH and LMH, as in this series of 7 and 4 cases respectively.

Thus for all VRI pathologies, routine surgical procedure involved PPV with double peel i.e. removal of ERM along with underlying ILM in this study. Rationale for removing ILM was to prevent recurrence of ERM. Reported incidence of recurrence is between 10 - 16.3% with reoperation rate of 3-5.8% in eyes undergoing ERM removal alone. This variable rate of ERM recurrence arises due to incomplete removal of ILM, which acts as a scaffold for glial proliferation after successful removal of the ERM. Also ERM is transparent or semitransparent. To facilitate complete removal, “double staining” technique using triamcinilone acetonide (TA) to stain the ERM followed by Brilliant Blue G (BBG) to stain the ILM was used in the present study. Mechanical microtrauma to neurosensory retina during ILM peeling and concern of vital dyes coming in contact with the retina after ERM removal have made it controversial. The safety profile of BBG has been well established with satisfactory anatomical and functional postoperative results. BBG reportedly damages Müller cells less than ICG and TB. Although this study did not use mfERG to rule out any adverse effect, favourable anatomical and visual outcome achieved validates the “double staining and double peeling” technique. Studies have shown that during ERM removal, the internal limiting membrane can be removed together with the ERM in about 40 – 60% of cases. Punctate retinal hemorrhages following ERM removal indicating simultaneous ILM peeling was found in 62.8% cases documented by Oh HN et.al. In present study we did not find this indirect evidence of simultaneous ILM peeling and moreover positive BBG stain following ERM removal indicates that instead of a “single-staining and single-peeling” procedure, restaining and peeling of the residual ILM is necessary. Also we did not find any recurrence of ERM or performed any resurgery in our follow up period of 6 months which although is a short period to report such an event. The mean follow-up period of all eyes in the current study was 6.5±1.3 months (range, 6-8 months). In a study comparing retinal distortion associated with ERMs, Arroyo and Irvine noted retinal relaxation within 6 days of membrane
removal. Yamamoto et. al. also noted retinal thickness reduction within 7 days postoperatively for patients who underwent vitrectomy, and Constantinidis et. al. found short-term 1-week visual recovery to be almost identical to long-term acuity in patients who had undergone 23-gauge ERM peeling. There were cases with a short follow up of 3 months only in the series by Kinoshita et. al. While postoperative macular remodeling and structural changes can affect visual recovery, in the current study, we believe that 6-month time was more than sufficient for retinal relaxation and remodeling in the retina, and therefore, the outcomes measured at the 6-month follow-up period should not significantly differ from those measured at later time frames.

Anatomical and Functional Outcome

Most of the preoperative demographic data did not significantly differ among the VRI types. Average preoperative CFT decreased in the following order: VMTS, ERM-, MPH-, and LMH-type VRI disorder. Meanwhile, preoperative vision was worse in the following order: ERM-, MPH-, VMTS, and LMH-type interface pathology. There was no significant correlation between preoperative CFT and BCVA, indicating that the magnitude of morphologic change in retinal layer did not affect vision in this series. Moreover, visual improvement was best in the ERM type, followed by the VMTS, MPH, and LMH types in that order, which is nearly consistent with that of mean CFT reduction. These findings are very similar to study of Kinoshita et. al. except that they did not differentiate between two types of non full thickness macular holes (NFMH).

In the current study, we reviewed each case to see whether combined PE/IOL or SF6 Tamponade would affect the postoperative outcome. Studies have shown no significant differences in outcomes in combined membrane peel and cataract extraction versus consecutive procedures. Dugas et. al. indicated that the functional and anatomical results of vitrectomy with membrane peeling combined with cataract extraction and lens implantation were equivalent to consecutive procedures. We, therefore, believed that concomitant PE/IOL procedure had no significant effect in our outcome analysis.

Epiretinal Membrane

Pars plana vitrectomy has been found to successfully remove ERMs from the macula resulting in an improvement in vision in 75% to 85% of eyes. In our study 84% eyes improved in visual acuity from 20/80 preoperatively to 20/32 postoperatively. This is similar to results shown in more recent studies by Mazit et. al., Pournaras et. al. and Panos et. al. Number of eyes improving by 2 lines (25/44.56%) and by 3 lines (13/44,29%) is again very similar to 36% reported in study of Fribourg Eye Clinic.
Visual acuity dropped by 1 line in a single patient and was stable in 6 eyes. This unexpected deterioration in a single patient can be due to microtrauma resulting in mechanical damage to the Müller cells and the structure of the fovea. OCT measurement of postoperative foveal thickness revealed a significant thickness decrease in our study (average reduction of 145µm). These findings are in agreement with other studies on removal of ERM with simultaneous ILM peeling using Brilliant Blue G and ICG.\textsuperscript{51,52} Lee and Kim in a comparative study found that thickening of the macula with loss of the normal foveal contour was more frequent in patients who underwent ERM and ILM peeling than in patients who underwent ERM peeling alone.\textsuperscript{18}

Also in the Swiss study\textsuperscript{50}, even at the end of one year, recovery of a normal foveal thickness was not achieved in the majority of cases as well as in study by Pournaras \textit{et. al.}\textsuperscript{51} In the present study there were 19 (43\%) eyes with persistent macular swelling at the end of six months. In all these eyes there was significant reduction in CFT from preoperative level which was much more thicker than the remaining eyes. This is an expected finding with a short follow up period. In our study and all of the above studies, final visual acuity improvement following idiopathic ERM removal along with ILM peeling was present despite restoration of completely normal foveal contour.

### Vitreomacular Traction Syndrome

The majority of published studies reported surgical outcomes associated with the VMT syndrome that indicate a high success rate with VA improvement in 44\%–78\% of cases.\textsuperscript{53,58} In this study we achieved a success rate of 94\%. In the earliest report by McDonald \textit{et. al.} which enrolled a series of 26 eyes, 75\% demonstrated improvement in VA of at least two Snellen lines, with eight achieving 20/50 VA or better.\textsuperscript{53} In a lately retrospective study conducted by Toklu \textit{et. al.}, the mean central macular thickness was 429 µm pre-operatively, which was significantly reduced to 255 µm at the 7-month follow-up visit with better VA post-operatively.\textsuperscript{58} Similarly, Koerner \textit{et. al.} observed a larger series of 50 eyes, and achievement of postoperative VA of 20/50 or better was demonstrated only in 66\% eyes with preoperative VA better than 20/100.\textsuperscript{59} Recently, in a retrospective review by Witkin \textit{et. al.}, of 20 eyes with VMT that had undergone vitrectomy with ERM peeling, mean VA improved from 20/122 before surgery to 20/68 following the operation.\textsuperscript{54} In this series there was a significant improvement in visual acuity from baseline 20/63 to postoperative 20/32. 13 (76\%) eyes had improvement in one line and 3 eyes improved by 2 lines or more. Only 1 eye did not improve functionally from 20/80, however there was significant reduction in CFT from 389µm to 211 µm post-operatively. Overall average CFT reduction was to the tune of 158 µm. This result is in accordance with the above studies.
Macular Pseudohole

In the earliest study by Massin et. al. looking at the outcome of surgery for ERM with and without pseudoholes, vision improved by 2 or more lines in 31 (62%) of 50 eyes with pseudoholes. Forty eyes (80%) reached visual acuity of 20/50 or more. Pseudohole persisted in 22 eyes (44%) 3 months after surgery and in 15 eyes (30%) at 6 months. There was no difference in visual acuity, whether or not the pseudohole persisted. This study observed an improvement of 2 or more lines in 71% (5/7) eyes with four eyes (57%) gaining visual acuity 20/40 or better. Pseudohole still persisted in 3 (42%) eyes at 6 months, but functionally having good visual outcome. This again could be due to the time which the macula takes to come to normal configuration following surgery. But with release of traction, the outer layers of retina helps in visual improvement. One eye showed paradoxical response of increase in macular thickness (390µm) at 3 months and eventually going into LMH configuration at 6 months with 206µm CFT. This was accompanied with visual deterioration to 20/80 from 20/63 preoperatively. This is in contrast to the study by Gaudric et. al. who concluded that macular pseudoholes with a stretched foveal edge respond to ERM peeling as positively as other eyes and do not progress to LMH. In their study of 33 eyes, macular profile and visual acuity improved irrespective of their initial foveal profile following vitrectomy.

Lamellar Macular Hole

Published reports of surgical treatment are limited, and they show variable results. Witkin et. al. performed vitrectomies in four eyes with LMH; only one eye had improved vision and foveal contour, whereas two eyes developed full-thickness macular holes after the procedure. Garretson et. al. achieved favorable anatomical and functional results in a series of 27 patients followed for a mean duration of 9 months, as did Engler et. al. in a series of 10 patients followed for a mean duration of 15 months. More recent studies confirmed the postoperative stability of the macular contour, as well as the VA following surgery. Our study revealed following surgery although vision improved in all cases by one line only but anatomically we could achieve normalized foveal contour and possibly arrested progression of LMH as reported by Theodossiadis et. al. This study demonstrated the advantages of vitrectomy in preventing natural worsening of the LMH diameter and foveal thinning and in protecting the inner foveal layer.

**CONCLUSION**

Introduction and widespread availability of modern technology, SDOCT for instance, not only promote unprecedented imaging but also raise the need to redefine concepts which are not yet settled and often confusing
such as the VRI disorder. This study for the first time looked into various types of VRI abnormalities with regard to their surgical outcome following a standardized technique. Our study has several limitations, including its retrospective nature, small sample sizes in few subtypes and overall a very short follow-up period. Regardless of these deficiencies, we present the outcomes of patients undergoing vitrectomies for visually symptomatic VRI diseases. “Double staining and Double Membrane Peeling” appears to be a safe technique for VRI types associated with ERM as demonstrated. Brilliant blue stain again emerged as a good alternative to ICG, IFCG or TB in chromovitrectomy because of its remarkable affinity for the ILM, and lack of retinal toxicity. Additional ILM peeling showed its merit by not having a single recurrence of ERM. This study did lack in testing macular function using mfERG to rule out any microtrauma objectively. Epiretinal membranes respond functionally very well albeit normalization to normal foveal contour requires more than six months following surgery. After surgery for idiopathic ERMs combined with pseudohole, visual outcome is good but the latter disappears inconstantly without precluding vision. Overall surgical benefit of vitrectomy in LMH seems still inconclusive, but the surgery seems to be beneficial in restoring the foveal contour and arresting progression. Recent studies on “enzymatic vitrectomy” have shown improvement in VA and release of retinal traction without the need for pars plana vitrectomy in VMTS. However, symptomatic VMTS with concurrent ERM requires surgical intervention in the form of double layer removal which cannot be addressed with pharmacologic vitreolysis alone. Concurrent cataract surgery along with vitrectomy did not affect final outcome. Use of intraocular tamponade may seem appropriate preoperatively in some of the VRI pathologies, but does not provide any faster restoration of the foveal contour. A prospective study on a larger scale and with longer follow-up and assessment of macular function using mfERG is warranted to fully understand the pathomorphological changes following intervention.

REFERENCES


Proteomic Analysis of Vitreous and Aqueous Humor

Dr. Krishna R Murthy, Dr. Praveen Ramachandra Murthy

The pathophysiology of several eye diseases is poorly understood. Since the vitreous and aqueous humor are metabolic repositories of surrounding ocular tissues we decided to investigate the proteome of these two ocular fluids using high resolution mass spectrometry-based analysis.

MATERIALS AND METHODS

The vitreous samples for the proteomic analysis were obtained from five patients undergoing vitrectomy for macular hole, three patients with congenital cataract who underwent cataract surgery with intra-ocular lens implantation and primary posterior capsulotomy and two samples from patients with traumatic cataract with undisturbed vitreous and intact lens capsule but who also needed vitreous surgery due to zonular damage. All samples were collected by pars plana vitrectomy, were centrifuged at 13,000 rpm at 4°C for 15 minutes and archived at −80°C until further use. The samples were pooled and depleted of abundant proteins. The depleted sample was subjected to multiple fractionation techniques such as SDS-PAGE followed by in-gel digestion and in-solution digestion followed by SCX chromatography (Strong cation exchange) and OFFGEL fractionation before further analysis by LC-MS/MS.

The aqueous humor samples were collected from 250 patients undergoing cataract surgery by paracentesis. The patients with any intercurrent ocular pathology were excluded from the study. The aqueous humor samples were pooled and abundant proteins were depleted using human multiple affinity removal system (MARS) spin cartridge 14 (Agilent Technologies Inc., Cat# 5188-6560). Two hundred and fifty micrograms of protein was resolved by SDS-PAGE and stained with Coommassie Brilliant Blue. Twenty six gel bands were excised and in-gel digestion was carried out. The extracted peptides were dried and stored at -80°C until LC-MS/MS analysis. 800 micrograms of proteins was subjected to in-solution digestion. Briefly, 800 µg of protein was reduced with 5 mM DTT (dithiothreitol) and alkylated using 10 mM IAA (indoacetamide).

The proteins were then digested with trypsin (modified sequencing grade; Promega, Madison, WI) at 37°C for 16 hours. The reaction was stopped with 0.1% formic acid. The peptides were dried, reconstituted with 0.1% trifluoroacetic acid. The digested peptides were then subjected to SCX chromatography and basic pH reversed phase liquid phase chromatography (bRPLC) before further analysis by LC-MS/MS.
Data analysis: Mass spectrometry data was processed using the Proteome Discoverer software (Version 1.4.1.14, Thermo Fisher Scientific, Bremen, Germany) for the vitreous samples and Proteome Discoverer (Version 1.3.0.339, Thermo Fisher Scientific, Bremen, Germany) for the aqueous samples. Mascot, SEQUEST and X! Tandem search engines were employed to maximize the peptide identification. The mass spectrometry data was searched against NCBI Ref Seq 59 for the vitreous data and Ref seq 50 for the aqueous data. We carried out a bioinformatics analysis of subcellular localization, molecular function and biological processes by searching the identified proteins against the manually curated Human Protein Reference Database (HPRD; http://hprd.org) and Human Proteinpedia (http://humanproteinpedia.org).

RESULTS

LC-MS/MS analysis of the vitreous samples resulted in the identification of 7,642 peptides from 1,205 proteins. When compared to previous reports, 682 proteins out of 1,205 were found to be uniquely identified in this study. LC-MS/MS analysis of aqueous humor samples resulted in the identification of 5,225 peptides corresponding to 818 proteins. When compared to previously published reports, 447 out of the 818 proteins were found to be uniquely identified in aqueous humor in this study. Comparison of aqueous humor proteins identified in this study with the proteins annotated in the Plasma Proteome Database confirmed that 668 out of 818 proteins have been previously reported in the human serum or plasma.

Classification of the vitreous proteins based on gene ontology

Of the 1,205 proteins identified in this study, 599 proteins possess signal peptides and 318 proteins are reported to be localized in extracellular compartment. The majority of the proteins reported in our study were localized to extracellular space (24%), cytoplasm (20%) or plasma membrane (14%). Classification based on molecular function showed that the large majority of the proteins are involved in catalytic activity (27%), structural activity (10%), binding activity such as calcium ion binding, receptor binding and complement binding (10%), cell adhesion molecule activity (4%), and transporter activity (4%). A large group (19%) of proteins are still unclassified in terms of their molecular function. Further categorization was done for biological processes which constitute metabolism (28%), cell communication (19%), cell growth (12%), and immune response (7%).

Classification of aqueous humor proteins using Gene Ontology

Of the 818 proteins identified in our study, 304 have signal peptides (SP), 46 contain transmembrane domain and 75 proteins contain both a signal
peptide and a transmembrane domain. Thirty-three percent of the identified proteins were localized in the extracellular matrix. Cytoplasmic proteins also constitute a similar percentage (~33%) of the total. The remaining proteins were distributed between the various organelles namely, the cellular membranes (15%), the nucleus (11%), the endoplasmic reticulum, Golgi and mitochondria (~ 2-3% each). A majority of the proteins were found to be involved in cell communication and signal transduction, followed by cell growth, differentiation, proliferation and energy pathways.

**CONCLUSION**

Our study provides an in-depth analysis of the vitreous and aqueous humor proteome in humans. Some of the proteins identified may serve as potential molecules of interest in better understanding some of the pathologic conditions such as diabetic retinopathy, glaucoma, cataract and uveitis.

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**Imaging Photoreceptor Density in Rod-Cone Dysfunction in Indian Population Using Adaptive Optics**

**Dr. Anjani Khanna, Dr. Rajani R Battu, Dr. Supriya Dabir, Dr. Rohit Shetty**

The human retina is a uniquely accessible tissue and has been evaluated by various imaging modalities like fundus photography, optical coherence tomography (OCT), fundus fluorescein angiography (FFA), scanning laser ophthalmoscopy (SLO) etc. The emergence of OCT in 1991 came as a breakthrough in retinal imaging and changed the way various retinal pathologies like macular edema were graded and treated. OCT at its highest resolution can image the photoreceptors in vivo but the resolution achieved in OCT similar to other imaging modalities is hampered by eye’s optical aberrations.

Adaptive optics is a technology primarily used to remove the atmospheric blur in astronomical telescopes. Its first application in ophthalmology was in the year 2000 to demonstrate cone photoreceptors in living human retina.

**Principle**

Adaptive optics compensates for the higher order aberrations originating from cornea and lens of the eye and gives lateral resolution up to 2 µm which
gives high quality images of photoreceptors, ganglion cells, capillaries etc., hence enabling in vivo microscopic evaluation of normal and diseased retina.

It comprises of a wavefront sensor (Shack-Hartmann sensor) to measure the eye’s wavefront aberrations and a wavefront corrector (deformable mirror) to correct for these aberrations allowing diffraction limited imaging of the retina.

Adaptive optics technology can be combined with OCT, SLO or fundus camera. Flood illuminated fundus camera captures images with brief imaging exposure time giving high resolution without the effects of eye motion. AO-OCT with the present systems has shown to provide ultrahigh 3D resolution (3*3*3 µm³) and ultrahigh speed images of the retina with the added advantages of improved lateral and axial resolution, reduced granular artifacts and increased sensitivity to weak reflections. AO-SLO allows for real time confocal imaging.

After its first use to demonstrate cone photoreceptors, adaptive optics has been used to study various other cells of the retina like retinal pigment epithelial cells, ganglion cells and leukocytes travelling in retinal capillaries. The retinal nerve fibre layer, retinal vessel wall and lamina cribrosa can also be visualized with adaptive optics technology.

We aimed at imaging and counting the cone photoreceptor density and distribution in patients with rod-cone dysfunction using Adaptive Optics.

**MATERIALS AND METHODS**

Twenty six eyes of twenty six patients were studied. All patients with clinical and electrophysiological diagnosis of rod cone dysfunction were included. Children, patients who were not able to perform the test, patients with profound visual loss and hence inability to fixate on the target for the test were excluded. Images were acquired through flood illuminated fundus camera with adaptive optics technology rtx-1 (Imagine Eyes, Orsey, France). Five images were acquired for each eye- foveal, nasal, temporal, superior and inferior and analysed using custom software.

**RESULTS**

The cone count and spacing were analysed and compared to age and sex matched controls. The counts were also compared between quadrants. Both Cone Count and Spacing were found to be significantly reduced when compared to the Normal Subjects.

The Cone Count and Spacing were compared for each of the quadrants and 2 degrees and 3 degrees. Freidman’s Anova was applied based on the
tests of Normality and p<0.05 was considered significant. Although the difference in Cone Count between all the four quadrants was found to be statistically significant but significant difference was noted only between temporal and nasal; and temporal and inferior (p<0.008 considered as significant). Spacing did not show any statistical significance between the quadrants. The cone count was found to be significantly different between 2 and 3 degrees. (p<0.01). Although, spacing did not show any statistical significance.

**DISCUSSION**

Inherited retinal degenerations affect about 1:2000 to 1:7000 people worldwide. These are a group of disorders like Retinitis Pigmentosa, Usher’s syndrome, Cone-rod dystrophies which are characterized by slowly progressive death of rod and cone photoreceptors and relentless vision loss. There is presently no definitive treatment for inherited retinal degenerations. The treatment options currently under trials are stem cells, gene therapy, neuroprotective drugs and retinal prosthesis.

The limitation to the development of these modalities has been lack of sensitive outcome measures of disease progression. Tests of visual function cannot be used as outcome measures as significant photoreceptor loss must have occurred before reliable significant differences are measurable in visual function. Objective sensitive measures of photoreceptor survival may reduce the time to identify a treatment effect of an experimental therapy.

**Applications of adaptive optics in inherited retinal degenerations**

In the extensive work done in recent years on adaptive optics early clinical applications have been focused on inherited retinal degenerations due to the easy visualization of photoreceptor by AO due to their strong optical wave-guiding behavior.

Various groups have studied the cone photoreceptor structure and their functional correlation in inherited retinal disorders. Chen et al. used
AO-SLO, SD-OCT and fundus guided microperimetry to study macular cone structure, lipofuscin deposition and visual function in patients with Stargardt disease and found a correlation with abnormal autofluorescence and abnormalities of cone morphology and packing on AO with corresponding impaired function.\textsuperscript{11}

Tojo \textit{et. al.} found a correlation between AO images, OCT and fundus autofluorescence images. They demonstrated an abnormal parafoveal ring of high density autofluorescence in 2 patients with retinitis pigmentosa (RP) the border of which corresponded to the border of external limiting membrane and inner segment-outer segment (IS-OS) line on OCT. On AO images these areas showed blurring of cone photoreceptors at the ring as compared to controls which they attributed to loss of photoreceptor outer segments in patients with RP.\textsuperscript{12}

Other studies have demonstrated the correlation of cone parameters on AO images with measures of central visual function including visual acuity, foveal threshold and multifocal ERG in patients with RP and Cone-rod dysfunction. Cone spacing measures were also seen to be reproducible suggesting that these can be useful in monitoring disease progression and response to treatment.\textsuperscript{13,14}

Variations in photoreceptor reflectivity have been observed in different disease states. Photoreceptor reflectivity in AO appears to represent an optical biomarker of photoreceptor integrity. In patients with achromatopsia the remaining cones are sparse and have been shown to have diminished reflectivity. Similar cone phenotype is also seen in patients with opsin mutations, acute macular neuroretinopathy (AMN) and closed globe blunt ocular trauma.\textsuperscript{15}

Rod and cone photoreceptors have been also shown to vary in intensity over time and by developing methods to quantify this temporal variability more insight into the health of photoreceptors can be available.\textsuperscript{16}

We found a significant reduction in cone densities in patients with rod cone dystrophies as compared to normal similar to other studies. Due to the lack of treatment modalities available for our patients with these diseases, adaptive optics presently will offer no additional benefit apart from providing information regarding the site and severity of cone loss but it can form the basis for selecting which patients will benefit most from therapy.
in future and provide an immediate measure of response to treatment. For example AO imaging of achromatopsia has revealed varying degrees of retained cone structure. Studying the structure and function of retained cones can set a baseline as to which patients will benefit from gene therapy (as has been successful in dog and mouse models) and which patients wont and what degree of functional improvement can be expected based on the patient’s baseline cone count. Hence it can differentiate which patients to undergo trial of gene therapy (i.e. those with certain number of retained cones) and which patients should not (those with absent cones). Also in a study by Talcott et. al. AOSLO was used to longitudinally study the cone spacing and density in 3 patients implanted with Ciliary Neurotrophic Factor (CNTF) encapsulated implant. While other modalities like visual field sensitivity and ERG responses did not show significant changes at 24 months, AOSLO images showed significantly reduced rates of cone loss in CNTF treated eyes. Longitudinal imaging and monitoring of individual cones was also done and they concluded that AOSLO provide a sensitive measure of disease progression and treatment responses in patients with retinal degenerations.

AOSLO can be used to deliver stimuli to individual cones and measure visual function at higher resolutions, this technique is still under development for retinal degenerations.

Limitations of adaptive optics

Patients with retinal diseases with disease severity resulting in unstable fixation precludes acquisition of AO images of cones of sufficient quality to perform quantitative cone spacing measures. Other modalities such as AF and SD-OCT have been seen to be more useful in such cases. Also eyes with dense cataracts, other media opacities and pseudophakia cannot be imaged.

The activation energy (cost and expertise) is quite high to access AO technology limiting its widespread use and acceptability.

As adaptive optics imaging is finding more clinical applications, normative databases need to be developed as a reference for disease states. Universally acceptable standards and protocols need to be defined for image acquisition and analysis to ensure reproducibility and comparability worldwide.

More studies need to be done on the reliability and repeatability of AO.

**CONCLUSIONS**

Adaptive optics is a safe non-invasive technique with enormous potential to explore retina at a cellular level. Its applications include investigation of retinal disease mechanism, longitudinal monitoring of disease progression,
early disease detection, providing finer end points for clinical trials and assessing response to treatment. As more research is ongoing on gene replacement therapy adaptive optics ophthalmoscopy could enable the gene replacement therapy, to be targeted to retinal locations with the most preserved photoreceptor mosaic, thereby directly increasing the potential for success of these therapies and subsequently monitor the response to treatment by directly examining the photoreceptor survival rates.

REFERENCES


SD-OCT Findings in Patients with Chorio-Retinal Coloboma in Different Age Groups: A Comparison

**Dr. Deepali Fauzdar, Dr. Alok Sen, Dr. Shubhi Tripathi, Dr. Ashish Mitra**

Chorio-Retinal Coloboma (CR-coloboma) is a congenital defect, results from incomplete closure of the embryonic fissure of neuroectodermal optic cup, which normally closes around sixth week of gestation. Any insult (genetic or environmental) occurs during 5th-8th week of gestation, cause defective closure of embryonic fissure and development of CR-coloboma occurs.\(^1,2,3\)

CR-colobomata are frequently associated with microphthalmia, microcornea, iris coloboma and lens coloboma. They typically occur in inferonasal quadrant. The extent of CR-coloboma can vary from small visually insignificant defect near the equator to large CR-coloboma involving entire disc and macular area with severe visual impairment.\(^4,5,6\)

Histopathologically, the area of CR-coloboma lacks normal choroid, Retinal pigment epithelium, while the underlying sclera is covered by intercalary membrane (ICM), which is hypoplastic and gliotic neurosensory retina.\(^7\)

Risk of retinal detachment is higher in eyes with CR-colobomata as compared to general population and the risk is maximum in young patients (<40 year) with CR-colobomata.\(^8\) Various morphologic features of the CR-colobomata and its margin previously studied on OCT scan shows association with risk of retinal detachment.\(^9\) In a histopathologic study, done by Schubert, there is a proven difference between the histology of CR-coloboma in pediatric eyes and adult eyes.\(^7\)
We used spectral domain optical coherence tomographic scans (SD-OCT) as a tool to look for differences in the morphology of CR-colobomata in different age groups. To the best of our knowledge this is the largest series evaluating the OCT features of CR-colobomata, and the first study of its kind, which compares the features of CR-colobomata in different age groups.

The purpose of this study is to report various morphologic features of CR-colobomata as seen on SD-OCT scan and to compare these features and their relative frequencies in different age groups.

**MATERIALS AND METHODS**

Present study was an observational case series of consecutive patients presented in our institute between July 2013 to April 2014 with CR-colobomata.

After approval from the institutional ethical board, all the patients with CR-colobomata underwent complete ophthalmologic examination. Patient’s demographic characters, age, sex, laterality were noted, colour fundus photographs were taken and SD-OCT image through the margin of colobomata (preferably near the macular area) were taken.

Inclusion criteria for our study was all the patients with CR-colobomata presented to our Institute during study period in which recording of good quality OCT image was possible, irrespective of nystagmus, microophthalmos, micro-cornea, corneal opacity and lenticular opacity. We excluded the patients with CR-colobomata in which obtaining good quality OCT image was not possible due to any reason such as gross nystagmus, significant corneal or lenticular opacity etc.

Patient’s demographic characteristics, colour fundus photographs and SD-OCT images were analysed. Classification of CR-colobomata was done in type-1,2,3,4 and 5 according to Idamann’s classification (Figure 1). On OCT, analysis of findings such as transition of coloboma margin from normal retina to inter-calary membrane (ICM) (abrupt or gradual) (Figure 2), presence or absence of inter-calary membrane detachment (ICMD) (Figure 3), presence or absence of cystoids spaces in ICM or in the retina near the edge of CR-colobomata (Figure 4), presence of subclinical sub-retinal fluid and schisis were noted. Statistical analysis was done using Pearson’s chi square test and p-value less than 0.05 was considered significant.

**RESULTS**

Under the study protocol total 360 eyes of 239 patients were enrolled and these underwent further analysis. Depending on their age patients were
Table 1: Distribution of patients in different age groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total Patients</th>
<th>Total eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-1</td>
<td>&lt;14 Years</td>
<td>28</td>
<td>20</td>
<td>48</td>
<td>73</td>
</tr>
<tr>
<td>Group-2</td>
<td>14-40 Years</td>
<td>59</td>
<td>60</td>
<td>119</td>
<td>180</td>
</tr>
<tr>
<td>Group-3</td>
<td>&gt;40 Years</td>
<td>41</td>
<td>31</td>
<td>72</td>
<td>107</td>
</tr>
<tr>
<td>Total Patients</td>
<td>128</td>
<td>111</td>
<td>239</td>
<td>360</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Laterality of CR-coloboma among the age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total no. of pts</th>
<th>No. of Pts with B/L Coloboma</th>
<th>No. of pts with U/L coloboma with other eye normal</th>
<th>Functionally one eyed patients with coloboma</th>
<th>Total no. of coloboma-tous eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14 years</td>
<td>48 (100%)</td>
<td>22 (45.8%)</td>
<td>3 (6.2%)</td>
<td>23 (48%)</td>
<td>70</td>
</tr>
<tr>
<td>14-40 years</td>
<td>119 (100%)</td>
<td>45 (37.8%)</td>
<td>16 (13.4%)</td>
<td>58 (48.7%)</td>
<td>164</td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>72 (100%)</td>
<td>18 (25%)</td>
<td>17 (23.6%)</td>
<td>37 (51.4%)</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>239 (100%)</td>
<td>85 (35.6%)</td>
<td>36 (15%)</td>
<td>118 (49.4%)</td>
<td>324</td>
</tr>
</tbody>
</table>

Table 3: Distribution of type of CR-coloboma in different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total no. of pts</th>
<th>No. of Pts with B/L Coloboma</th>
<th>No. of pts with U/L coloboma with other eye normal</th>
<th>Functionally one eyed patients with coloboma</th>
<th>Total no. of coloboma-tous eye</th>
</tr>
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<td>18 (25%)</td>
<td>17 (23.6%)</td>
<td>37 (51.4%)</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>239 (100%)</td>
<td>85 (35.6%)</td>
<td>36 (15%)</td>
<td>118 (49.4%)</td>
<td>324</td>
</tr>
</tbody>
</table>

Table 4: Type of transition of CR-coloboma margin in different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No. of Eyes (%)</th>
<th>Gradual</th>
<th>Abrupt</th>
<th>Humped</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14 (Group-1)</td>
<td>70 (100%)</td>
<td>57 (81.4%)</td>
<td>11 (15.7%)</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>14-40 (Group-2)</td>
<td>164 (100%)</td>
<td>110 (67%)</td>
<td>46 (28%)</td>
<td>8 (5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;40 (Group-3)</td>
<td>90 (100%)</td>
<td>46 (51%)</td>
<td>35 (39%)</td>
<td>9 (10%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>324</td>
<td>213 (65.7%)</td>
<td>92 (28.4%)</td>
<td>19 (6%)</td>
<td></td>
</tr>
</tbody>
</table>

divided in three groups; group-1 included patients less than 14 year age, group-2 between 14 to 40 year age, and group-3 more than 40 year age. Group wise distribution of patients and their demographic details are given in Table 1.

Out of total 239 patients, 85 patients had bilateral CR-colobomata, 36 patients had unilateral CR-coloboma with other eye normal and 118 patients were
functionally one eyed with CR-coloboma in one eye and other eye with either extreme microphthalmos, anophthalmos or significant lenticular opacity due to which only one eye OCT was taken (Table-2).

So out of total 360 eyes examined 324 eyes were with CR-colobomata and 36 eyes were normal. Therefore further analysis was carried out in these 324 eyes with CR-colobomata.

**Type of Coloboma**

Out of total 324 eyes, type 1 CR-coloboma was found in 113 eyes, type 2 in 46 eyes and most common was type 3 in 155 eyes. Type 1 was most common in group 1 and type 3 most common in group 3. Group wise distribution of types of colobomata are given in table 3.

**Transition of coloboma margin**

Gradual ending margin was the most common type among all the 324 eyes found in 65.7% of eyes, as well as in individual group. But a uniform trend of decrease in percentage of eyes with gradual ending margins with increasing age and increase in percentage of eyes with abrupt ending margin with increasing age was seen (Table 4).

**Intercalary membrane detachment**

Intercalary membrane detachment was seen in 124 out of 324 eyes and it was most commonly seen in group 1 (Table 5).

### Table 5: Prevalence of Inter-calary membrane detachment in different age groups

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No. of Eyes (%)</th>
<th>ICMD Present</th>
<th>ICMD Absent</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14 years (Group-1)</td>
<td>70 (100%)</td>
<td>34 (48.6%)</td>
<td>36 (51.4%)</td>
<td></td>
</tr>
<tr>
<td>14-40 years (Group-2)</td>
<td>164 (100%)</td>
<td>64 (39%)</td>
<td>100 (61%)</td>
<td>0.015</td>
</tr>
<tr>
<td>&gt;40 years (Group-3)</td>
<td>90(100%)</td>
<td>26(29%)</td>
<td>64(71.1%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>324</td>
<td>124(38.3%)</td>
<td>200(61.7%)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 6: Cystoids spaces in the Inter-calary membrane/ in the retina near the margin of CR-coloboma

<table>
<thead>
<tr>
<th>Age group</th>
<th>Total No. of Eyes (%)</th>
<th>Cyst present</th>
<th>Cyst absent</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;14 (Group-1)</td>
<td>70 (100%)</td>
<td>43 (61.4%)</td>
<td>27 (38.6%)</td>
<td></td>
</tr>
<tr>
<td>14-40 (Group-2)</td>
<td>164 (100%)</td>
<td>80 (39%)</td>
<td>84 (51.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;40 (Group-3)</td>
<td>90 (100%)</td>
<td>25 (27.8%)</td>
<td>65 (72.2%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>324</td>
<td>148 (36%)</td>
<td>176 (54.3%)</td>
<td></td>
</tr>
</tbody>
</table>
Cystoid spaces in intercalary membrane and margin

Cystoid spaces denote some form of subclinical detachment, presence of traction and its chronic nature, were found in 148 eyes and they were more common in group 1 (Table 6).

Other features

Other important features on SD-OCT of CR-colobomata include presence of sub retinal fluid, which was found in 4 eyes in group 1, 14 eyes in group 2 and 4 eyes in group 3. Retinoschisis was seen in 2 eyes in group 2 and one eye in group 3.

DISCUSSION

Chorio-Retinal colobomata are frequently associated with retinal detachment (8.1–42%), that occurs more commonly in younger age group and is rarely seen after 40 years of age. Reported age for retinal detachment presentation in CR-colobomata are average between 19 to 26 years.\textsuperscript{7,10,11,12,13} Various characteristic features of CR-colobomata and their margins suggest the risk of development of retinal detachment. These features can be examined better by OCT examination.\textsuperscript{9,14} On comparing the different age groups we have found a difference in frequency of various risk factors in different age groups.

Among the groups, percent of patients with CR-coloboma in one eye with other eye normal was highest in older age group; group 3 with age more than 40 years (23.6%). These patients usually presented for their ocular complains unrelated to colobomata and detection of CR-coloboma was coincidental. The percent of patients in which only single eye OCT was recorded was also highest (51.4%) in group 3. The reason for only single eye OCT was significant lenticular opacity in the other eye, for which they had presented to our institute.

While in younger age groups (group 1 and 2) either bilateral CR-colobomata or one eye CR-coloboma with other eye microphthalmos, anophthalmos, extreme microcornea with corneal opacities were common, and they presented to our institute with their complains related to CR-colobomata.

If we look for distribution of type of colobomata in all groups, it is evident that in group 3, type 3 CR-colobomata were most common which are frequently asymptomatic and coincidental finding. While in group 1, type 1 CR-colobomata were most common and because of involvement of disc and macula it significantly affects the function of eye and therefore patient presents early. Also the chance of retinal detachment related to CR-colobomata are higher with type 1 coloboma.\textsuperscript{13}
In the area of CR-colobomata, choroid and RPE are absent and at the margin of coloboma neurosensory retina forms the intercalary membrane. This conversion from normal retina to ICM can be of two type as described by Gopal et.al. The first is gradual transition, in which outer layer of neurosensory retina is lost early and inner layers continue for some distance in coloboma area and then form featureless ICM. The second is abrupt transition in which neurosensory retina suddenly transforms to ICM. Gradual transition is more common as so in our study with overall 67.7% eyes had gradual transition and 81.4%, 67% and 51.1% eyes respectively in group 1, 2 and 3. 19 eyes were found to have humped edge, thickening of inner retina at margin, which suggest structural stability. Out of these 19 eyes 9 were in group 3.

In a histopathologic study, done by Schubert, it is described at the margin of CR-coloboma, a schisis like split occurs in neurosensory retina, inner layer continues towards centre of CR-coloboma by forming ICM and outer layer revert back to form junction with RPE at the margin of CR-coloboma and forms locus minoris resistentiae.
For the occurrence of CR-coloboma related retinal detachment along with break in ICM, intercalary membrane detachment is must. Presence of ICMD visible clinically or detected on OCT scan, is high risk factor for retinal detachment, and if present it should be addressed.\textsuperscript{9,14} In our study ICMD were found more commonly in group 1 and least commonly in group 3. This finding also suggests younger patients have more risk of developing retinal detachment related with CR-colobomata.

Even if there is presence of break in ICM and ICMD but no communication between sub-ICM and sub-retinal space, retinal detachment will not occur. This communication can develop by dehiscence in locus minoris resistentiae, by continuous traction of a stretched ICM.\textsuperscript{9,14}

Presence of cystoid spaces in ICM and in the retina near the margin also indicates continuous traction which can cause dehiscence and give way for sub retinal fluid, and therefore their presence is a risk factor for RD.\textsuperscript{9,14} In our study cystoid spaces were found more commonly in group 1 (61.4\%) than in other groups. Presence of sub clinical SRF carries high risk for RD and indicates sub clinical detachment which was also detected in 22 eyes, out of which 14 were from group 2.

This study is the largest series on OCT of patients with CR-colobomata and the first study with age wise comparison of OCT features of CR-colobomata. This is an observational study and we did not follow-up the patients over time, therefore we do not know about progression of these features, that how many of young eyes, with time convert to morphologic features more found in adults or remain stable. Also how many of these eyes will develop retinal detachment in future. To look for this we need a study with long term follow-up of patients.

To summarize, features of CR-colobomata like type 1 coloboma, gradual transition of margins, presence of ICM detachment and cystoid spaces, all suggest high risk of RD, as evident in previous studies, were seen more commonly in younger patients presenting with CR-colobomata as compared to older patients more than 40 year age. By careful examination of OCT scans we can find out these high risk patients to observe them closely and can perform prophylactic laser barrage of CR-coloboma to prevent development of RD. Even if OCT is not available, if a young patient with type 1 CR-coloboma presents with ICM detachment, it will be better to go for prophylactic barrage laser.

REFERENCES


