Vitreoschisis in macular diseases

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ABSTRACT

Objectives Vitreoschisis is a possible pathogenic mechanism in macular diseases. Thus, the vitreoretinal interface was evaluated in monkey eyes and patients with various macular diseases in search of vitreoschisis. It is hypothesised that vitreoschisis is present in macular holes (MH) and macular pucker (MP), but not in other maculopathies.

Methods Histopathology was studied in 14 monkey eyes and a vitrectomy specimen of a patient with macular pucker. Optical coherence tomography/scanning laser ophthalmoscopy (OCT/SLO) was performed in 239 eyes: 45 MH, 45 MP, 51 dry age-related macular degeneration (AMD), 53 non-proliferative diabetic retinopathy (NPDR) and 45 controls.

Results Immunohistochemistry demonstrated lamellae in the posterior vitreous cortex of 12/14 (86%) monkey eyes. With OCT/SLO, vitreoschisis was detected in 24/45 (53%) MH and 19/45 (42%) MP eyes, but in only 7/53 (13%) NPDR, 3/51 (6%) AMD and 3/45 (7%) control eyes ($p<0.001$ for all comparisons). Rejoining of the inner and outer walls of the split posterior vitreous cortex was visible in 16/45 (36%) MH eyes and 15/45 (33%) MP eyes. Histopathology of the MP specimen confirmed a split with rejoining in the posterior vitreous cortex.

Conclusions Vitreoschisis was detected in half of eyes with MH and MP, but much less frequently in controls, AMD and NPDR patients. These findings suggest that anomalous PVD with vitreoschisis may be pathogenic in MH and MP.

INTRODUCTION

The aetiologies of macular holes (MH) and macular pucker (MP) are poorly understood. Past theories of MH pathogenesis included ocular trauma, cystic degeneration and ocular angiospasm. More recent theories[1 2] propose that vitreous plays a predominant role in the formation of MH. Vitreous changes have also been implicated as pathogenic in MP. Since a high incidence of posterior vitreous detachment (PVD) is found in MP,[3 4] one theory proposes that PVD causes micro-breaks in the retina that allow for the migration of fibroblasts, glial cells and astrocytes from the retina to the surface of the internal limiting lamina where they proliferate.[5] However, such breaks have not been routinely detected on histopathological analysis.

A recent hypothesis is the unifying concept of anomalous PVD,[2] which proposes that collapse of the liquefied vitreous body without sufficient dehiscence at the vitreoretinal interface can induce various abnormalities at the vitreoretinal interface. One such abnormality is a split within the posterior vitreous cortex, known as vitreoschisis. Anomalous PVD with vitreoschisis can leave the outermost layer of the split posterior vitreous cortex attached to the macula.[6] Although vitreoschisis has been previously described in proliferative diabetic retinopathy,[7 8] there have been no comprehensive studies in macular diseases.

Recent advances in fundus imaging have made available optical coherence tomography combined with scanning laser ophthalmoscopy (OCT/SLO). With its improved resolution of the vitreoretinal interface, OCT/SLO has enabled exploration of eyes with macular diseases to search for vitreoschisis. This study employed OCT/SLO imaging of subjects with macular diseases and controls, histopathological analysis of tissue removed from the vitreoretinal interface of one subject with MP and immunohistochemistry of the vitreoretinal interface in monkey eyes to search for evidence of vitreoschisis.

PATIENTS AND METHODS

Institutional Review Board approval and informed consent were obtained.

Experimental subjects (macular holes and macular pucker)

Ninety (90) eyes from 82 subjects with the clinical diagnosis of MH (n=45) or MP (n=45) were evaluated between March 2005 and November 2008 at the VMR Institute in Huntington Beach, California (n=57) and the New York Eye and Ear Infirmary (n=33). All subjects were evaluated by slit-lamp biomicroscopy, indirect ophthalmoscopy, fluorescein angiography and combined OCT/SLO.

Macular hole

There were 18 men (40%) and 27 women (60%), with a mean age of 64.7±14.4 years. Stage II MHs were diagnosed in 6/45 (13.3%) patients, while 29/45 (64.4%) had stage III MHs, and 10/45 (22.2%) had stage IV MHs. The mean diameter of the holes was 586 μm with a SD of 251 μm. The demographics, mean diameter of the MHs, and the presence or absence of vitreoschisis (VS), are indicated in table 1.

Macular pucker

There were 26 men (57.8%) and 19 women (42.2%) with a mean age of 66.4±7.6 years. All eyes had visible retinal striae on physical examination, constituting grade 2 MP by Gass classification.[9] Longitudinal (B-scan) OCT/SLO showed distinct corrugation of the underlying retina, and the average foveal thickness by OCT/SLO topography was 303.2 μm with an SD of 107.5 μm (range 157–512 μm). The demographics, foveal thickness, and presence or absence of VS are indicated in table 1.

Table 1 Demographics, clinical characteristics and incidences of vitreoschisis in experimental subjects (macular hole and macular pucker)

	Age (years) (mean±SD)	Sex	Macular hole diameter (μm)	Foveal thickness (μm)	Vitreoschisis
Macular holes (n=45)	64.7±14.4	27 women (60%)	586±281		24/45 eyes (53.3%)
Macular pucker (n=45)	66.4±7.6	19 women (42%)		303±107	19/45 (42.2%)

Control subjects

Controls were all subjects recruited from the VMR institute in Huntington Beach, California with no eye disease (normals; 45 eyes in 36 subjects) as well as subjects with dry age-related macular degeneration (AMD; 51 eyes in 50 subjects) and non-proliferative diabetic retinopathy (NPDR; 53 eyes in 28 subjects).

Histopathological analyses

A subject with MP and vitreoschisis underwent 25-gauge vitrectomy with membrane peel (by JS). The peeled membrane was fixed in formalin, embedded in paraffin, and stained with H&E, periodic acid–Schiff and Alcian Blue glycosaminoglycan stain for microscopy.

Adult monkey eyes (n=14) were fixed in 4% paraformaldehyde, sectioned and stained with fluorescein-conjugated ABA lectin, as previously described.[10]

Optical coherence tomography/scanning laser ophthalmoscopy (OCT/SLO)

Fundus imaging was performed using the combined OCT/SLO (OPKO Instrumentation, Miami, Florida), which incorporated a dual-channel scanner linked to an interferometer and a confocal receiver. An infrared superluminescent diode with a central wavelength of 820 nm provided the light source used by the OCT/SLO. In the longitudinal (B-scan) mode, the OCT/SLO employed a Galvano-Scanning mirror system to move the beam transversely in successively deeper linear horizontal sweeps of the retina to create cross-sectional images. In the coronal (C-scan) mode, planar (x,y) images were captured by opposing pairs x and y axis Galvano-Scanners, which moved the beam in a raster fashion across the surface of the retina. Stacks for three-dimensional reconstruction could be generated by capturing these C-scan (x,y) planar images at a successive z-axis depth. The axial or depth resolution, which depends upon the light source, was approximately 10 μm, while the transverse resolution, which is limited by the optics of the eye, was approximately 20 μm.

Statistical analyses

The results in each group were compared with one another using the χ^2 test. In addition to the individual comparisons, the two experimental groups (MH and MP) were combined and compared with the AMD and NPDR controls groups combined, as well as with the normal controls in a separate analysis.

RESULTS

Immunohistochemistry of the vitreoretinal interface

Immunohistochemistry of the monkey vitreoretinal interface revealed the existence of lamellae in the posterior vitreous cortex adjacent to the internal limiting lamina of the retina in 12/14 (86%) eyes (figure 1).

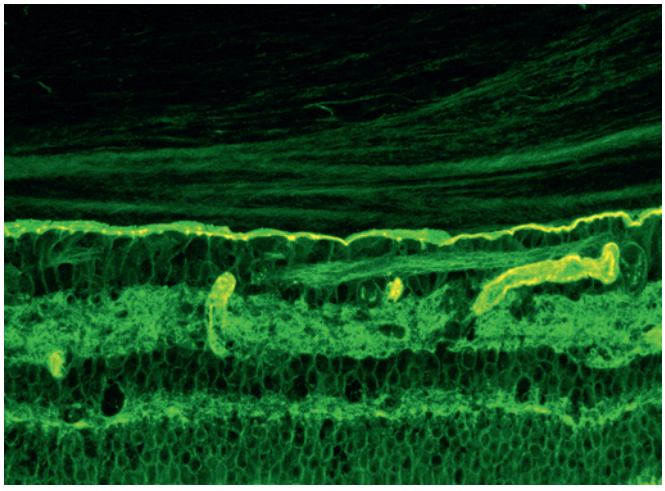

Figure 1 Immunofluorescence of the monkey vitreoretinal interface. The lamellae of the mammalian posterior vitreous cortex are seen anterior to (above) the intensely stained internal limiting lamina. (effective magnification ~400×).

OCT/SLO imaging of the human vitreoretinal interface

Vitreoschisis in macular holes and macular pucker

Vitreoschisis was considered definitely present when two membranous layers were seen to join into one, forming the shape of a 'lambda' or the letter 'Y.' By this criterion, OCT imaging detected vitreoschisis in 16/45 (35.6%) eyes with MH (figure 2) and 15/45 (33.3%) eyes with MP (figure 3). In other cases, there was no λ sign but visible evidence of a membrane on the posterior aspect of the detached vitreous, and a separate and distinct second membrane attached to the retina ('±' in tables 1, 2). By these two criteria, vitreoschisis was present in 24/45 eyes (53.3%) with MH, and 19/45 (42.2%) with MP.

Vitreoschisis in controls

There were 14 men (38.9%) and 22 women (61.1%), with a mean age of 62.64±10.9 years with no eye disease that served as normal age-matched controls. Of the 45 eyes studied, VS was detected in 3/45 (6.7%) eyes, and in all three cases there was a λ sign.

In the dry AMD control group, there were 20 men (40%) and 30 women (60%). VS was detected in 3/51 (5.9%) eyes, and in all three cases, there was a λ sign.

In the NPDR control group, there were 14 men (50%) and 14 women (50%). VS was detected in 7/53 (13.2%) eyes, three of which had a λ sign.

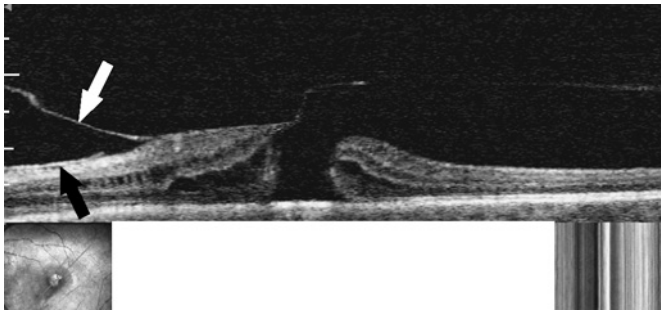

Figure 2 Vitreoschisis in macular hole. Longitudinal optical coherence tomography and scanning laser ophthalmoscopy (lower left hand corner) imaging of the vitreoretinal interface in an eye with a stage III macular hole demonstrates the inner (white arrow) and outer (black arrow) walls of the vitreoschisis that is readily apparent in this case.

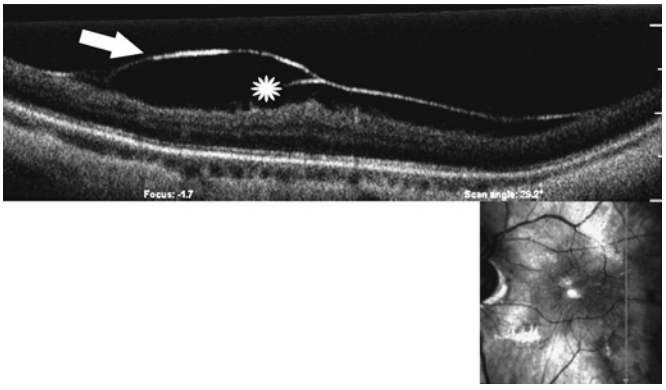

Figure 3 Vitreoschisis in macular pucker. Longitudinal optical coherence tomography and scanning laser ophthalmoscopy (lower right hand corner) imaging of the vitreoretinal interface in an eye with a grade II macular pucker demonstrates the two walls (inner wall=arrow; outer wall=asterisk) of vitreoschisis forming a 'lambda' sign where they rejoin.

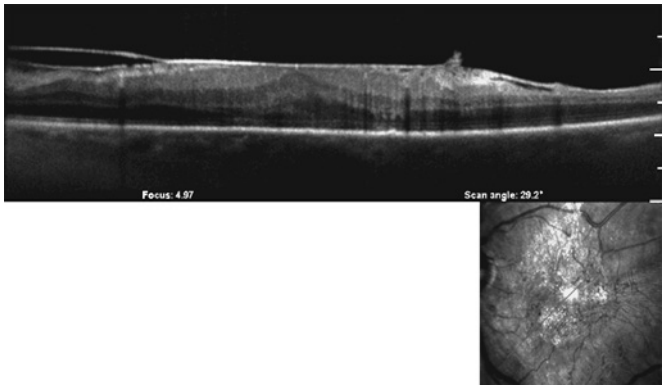

Figure 4 Optical coherence tomography/scanning laser ophthalmoscopy imaging of vitreoschisis in macular pucker. The left-hand side of the image demonstrates a split in the posterior vitreous cortex, or 'vitreoschisis.' The specimen was removed surgically (JS) and studied by histopathology (see figure 5).

Statistical analyses

Table 2 compares the findings in MP to each control group as well as the combined AMD and NPDR controls. The findings in MH were also compared with each control group as well as the combined AMD and NPDR controls. The combination of MH and MP was compared with normal controls and with the combination of AMD and NPDR controls. All comparisons were statistically significant ($p=0.001$ for MP vs NPDR controls, and $p<0.0001$ in all other comparisons).

Histopathology of the human vitreoretinal interface

Preoperative combined OCT/SLO imaging of the vitreoretinal interface in a 58-year-old male with MP is presented in figure 4. Vitreoschisis with rejoining of the two walls of the vitreoschisis cavity is clearly visible. Figure 5 shows the results of the histopathological analysis performed on the specimen removed at surgery in this individual. The periodic acid–Schiff stain demonstrated a discrete split in the posterior vitreous cortex, while the presence of hyalocytes and positive alcian blue staining of the same specimen confirm that the tissue that has undergone splitting is the posterior vitreous cortex.

DISCUSSION

As demonstrated herein, the posterior vitreous cortex comprises layers or lamellae that are tangential to the internal limiting lamina (ILL) of the retina. This lamellar structure represents an anatomical predisposition to cleavage between the lamellae, splitting the posterior vitreous cortex into separate layers,[6] a phenomenon known as vitreoschisis.[6–8] Vitreoschisis has previously been detected in proliferative diabetic retinopathy by ultrasound[7] and histopathology,[8] and in one case of vitreomacular traction syndrome by OCT.[11] Vitreoschisis was also described in 18 cases of Eales disease[12] and in a variety of retinovascular disorders.[13] While vitreoschisis has previously been suggested in myopic MHs, no objective clinical evidence has ever been presented for vitreoschisis in macular diseases, although recent observations during vitrectomy surgery described the intraoperative induction of vitreoschisis in 80% of eyes with MP.[14]

Table 2 Comparisons of experimental and control groups

Group	Incidences (%)	p Value
MP versus NPDR	42.2 versus 13.2	0.001
MP versus AMD	42.2 versus 5.9	<0.0001
MP versus normals	42.2 versus 6.7	<0.0001
MP versus NPDR+AMD	42.2 versus 9.5	<0.0001
MH versus NPDR	53.3 versus 13.2	<0.0001
MH versus AMD	53.3 versus 5.9	<0.0001
MH versus normals	53.3 versus 6.7	<0.0001
MH versus NPDR+AMD	53.3 versus 9.5	<0.0001
MP+MH versus NPDR+AMD	47.8 versus 9.5	<0.0001
MP+MH versus normals	47.8 versus 6.7	<0.0001

AMD, dry age-related macular degeneration; MH, macular hole; MP, macular pucker; NPDR, non-proliferative diabetic retinopathy.

In this study, high-resolution combined OCT/SLO imaging of the vitreoretinal interface detected vitreoschisis in half of eyes with MH and MP, but much less frequently in other conditions and in normal eyes. Histopathological analyses confirmed these clinical findings in one case. Although vitreoschisis was detected in both MH and MP, there are distinct clinical differences between these two diseases which must result from other factors. Recent studies[15] demonstrated a much higher incidence of vitreopapillary adhesion in eyes with MH as compared with MP, possibly explaining the differences.

Another explanation could be that there is a difference in the level at which the vitreoschisis split occurs during anomalous PVD.[6] Embedded within the posterior vitreous cortex are hyalocytes, which are mononuclear phagocytes located in a single layer approximately 50 μm from the ILL of the retina.[16–18] If the plane of the vitreoschisis split is posterior to the level of hyalocytes, these cells will detach away from the retinal surface along with the anterior portion of the split vitreous cortex. This probably happens in MH, leaving a relatively hypocellular, thin layer of vitreous attached to the macula. Intraoperative observations at surgery for MH confirm that these membranes are relatively thin and difficult to engage, often requiring the use of deeper dissection ('ILM Peeling') and/or chromodissection.[18] Histopathological studies[19] have found native vitreous collagen attached to the internal limiting lamina in 36 out of 100 cases of MH, an incidence quite close to that observed by OCT/SLO in this study. Furthermore, these membranous vitreous remnants were found more often in cases that had preoperative PVD, as compared with surgically induced PVD, suggesting a role in MH pathogenesis and supporting the concept of anomalous PVD.[2]

In MP, the vitreoschisis split likely occurs more anteriorly, leaving hyalocytes attached to the macula. As mononuclear phagocytes of the reticulo-endothelial cell system, 'sentinel'

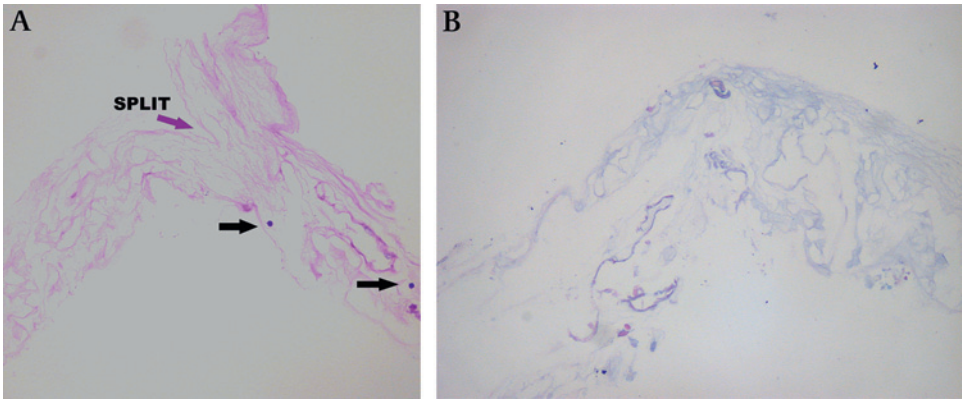

Figure 5 Histopathology of vitreoschisis in macular pucker. (A) Periodic acid—Schiff staining showing the split (purple arrow) in the vitreous membrane removed at surgery. Embedded in this tissue are two hyalocytes (black arrows), consistent with the concept that this tissue is the posterior vitreous cortex (courtesy of N Rao, MD; magnification=225×). (B) Surgical specimen (same subject as that in (A)) showing positive staining with Alcian Blue, confirming its origin as the posterior vitreous cortex (courtesy of N Rao, MD; magnification=300×).

hyalocytes can stimulate the migration of monocytes from the circulation and glial cells from the retina. Recent studies[20] have shown that hyalocyte metabolism can indeed be influenced by cytokines, which may also induce further cell proliferation. The proliferation of these cells on the surface of the retina results in hypercellular membranes that are thicker than those found in MHs. Indeed, studies[21] have shown that hyalocytes may play an important role in proliferative disorders of the retina via the release of connective tissue growth factor. Other studies[22] have furthermore shown that hyalocytes can induce collagen gel contraction in response to platelet-derived growth factor and other cytokines. Thus, hyalocytes are likely to be important not only in stimulating cell proliferation, but also in inducing tangential vitreoretinal contraction, the two main components of MP.

Vitreoschisis may also represent a cogent explanation for the recurrences seen following surgery for MH and MP. If only the anterior layer or inner wall of the vitreoschisis cavity is removed at surgery, the posterior layer, or outer wall of the vitreoschisis cavity, will remain on the anterior surface of the retina. Studies in MH surgery have shown that aggressive chromodissection[18] is associated with a lower rate of recurrent disease.[23 24] It is likely that with aggressive chromodissection of the vitreoretinal interface, the outer wall of the vitreoschisis cavity is definitely removed, decreasing the rate of recurrence. Studies using 'ILM peeling' in MP surgery also show a more favourable outcome than those without aggressive membrane dissection.[25]

In conclusion, the findings of this study support the hypothesis that vitreoschisis occurs in MHs and MP, probably as a result of anomalous PVD. That only half of eyes had OCT/SLO evidence of vitreoschisis is due either to the existence of more than one mechanism of disease, or that current imaging technology cannot resolve structures as small as some of the lamellae in the posterior vitreous cortex (figure 1). The future use of higher-resolution imaging should confirm whether the tissue planes detected in these studies are indeed lamellae within the posterior vitreous cortex or whether the internal limiting lamina of the retina is somehow also involved. More advanced imaging modalities may also help ascertain whether the incidence of vitreoschisis is even greater than that identified in this investigation. Future studies should also be undertaken to correlate histopathological analysis of tissue removed at surgery with clinical and OCT/SLO findings, so as to determine whether the cellular compositions are consistent with the differences noted on clinical evaluation, imaging, and the hypotheses presented herein.

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Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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