Original Article

Scanning electron microscopy of the trabecular meshwork: Understanding the pathogenesis of primary angle closure glaucoma

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Purpose: To study ultrastructural changes of the trabecular meshwork in acute and chronic primary angle closure glaucoma (PACG) and primary open angle glaucoma (POAG) eyes by scanning electron microscopy. **Materials and Methods:** Twenty-one trabecular meshwork surgical specimens from consecutive glaucomatous eyes after a trabeculectomy and five postmortem corneoscleral specimens were fixed immediately in Karnovsky solution. The tissues were washed in 0.1 M phosphate buffer saline, post-fixed in 1% osmium tetraoxide, dehydrated in acetone series (30-100%), dried and mounted. **Results:** Normal trabecular tissue showed well-defined, thin, cylindrical uveal trabecular beams with many large spaces, overlying flatter corneoscleral beams and numerous smaller spaces. In acute PACG eyes, the trabecular meshwork showed grossly swollen, irregular trabecular endothelial cells with intercellular and occasional basal separation with few spaces. Numerous activated macrophages, leucocytes and amorphous debris were present. Chronic PACG eyes had a few, thickened posterior uveal trabecular beams visible. A homogenous deposit covered the anterior uveal trabeculae and spaces. Converging, fan-shaped trabecular beam configuration corresponded to gonioscopic areas of peripheral anterior synechiae. In POAG eyes, anterior uveal trabecular beams were thin and strap-like, while those posteriorly were wide, with a homogenous deposit covering and bridging intertrabecular spaces, especially posteriorly. Underlying corneoscleral trabecular layers and spaces were visualized in some areas. **Conclusions:** In acute PACG a marked edema of the endothelium probably contributes for the acute and marked intraocular pressure (IOP) elevation. Chronically raised IOP in chronic PACG and POAG probably results, at least in part, from decreased aqueous outflow secondary to widening and fusion of adjacent trabecular beams, together with the homogenous deposit enmeshing trabecular beams and spaces.

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Primary angle closure glaucoma (PACG) has been recognized to be a significant cause of irreversible blindness in the world.[1] Its pathomechanisms are being studied, however, there is still very little known about histopathological changes in the trabecular meshwork that cause the rise in intraocular pressure (IOP). There have been a few case reports[2,3] and one comparative study on light and transmission electron microscopic findings in the trabecular meshwork of eyes with acute and chronic PACG.[4]

Alvarado *et al.*, noted that loss of human trabecular cells by phagocytosis or otherwise, commonly occurs closest to the anterior chamber, in the uveal trabeculae, and leads on to trabecular dysfunction.[5] The hallmark of primary angle closure is evidence of peripheral iridocorneal contact – apposition or adhesion, i.e. peripheral anterior synechiae or clumps of pigment in an occludable angle on gonioscopy, closest to the anterior chamber, on the intracameral face of the trabecular meshwork.

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We therefore studied the intracameral surface of the trabecular meshwork using scanning electron microscopy (SEM), in eyes having PACG, both acute and chronic, as compared to primary open angle glaucoma (POAG) and normal eyes.

Materials and Methods

Consecutive adult patients having POAG, acute PACG or chronic PACG who were undergoing a trabeculectomy for medically uncontrolled IOP underwent a thorough clinical examination, including visual acuity, slit-lamp evaluation, tonometry, fundus examination and perimetry.

Inclusion criteria for this study were POAG: an IOP > 21 mm Hg on three random recordings with Goldmann applanation tonometry, in the presence of open angles on gonioscopy, and characteristic optic nerve head and visual field changes of glaucoma. Chronic PACG: patients were diagnosed as having chronic PACG if a chronically raised IOP of over 21 mm Hg, on at least three occasions was recorded, in the presence of optic nerve head and visual field changes suggestive of glaucoma. An 'occludable' angle with peripheral anterior synechiae extending over at least 180 degrees had to be documented on indentation/manipulative gonioscopy. Patients with a prior history of acute angle closure were excluded. Acute angle closure glaucoma: patients with acute PACG had to have at least two of the following symptoms of a congestive episode—a severe unilateral headache, painful red eye, diminution of vision with or without nausea/vomiting. On examination, a

markedly raised IOP (> 40 mm Hg) in an eye with a shallow anterior chamber, vertically oval mid-dilated pupil, marked corneal edema and a closed angle had to be present, with or without glaucomatous optic neuropathy.

Eyes were excluded from the study if there was a history or signs of any other ocular pathology, prior surgery or trauma. Specimens were excluded if trabecular meshwork could not be identified.

The study was approved by our Institutional ethics committee, and an informed consent was obtained. The work was conducted within the tenets of the Declaration of Helsinki.

All patients underwent a careful preoperative examination, including slit-lamp examination, +90D fundus evaluation, perimetry and diurnal phasing on therapy. A gonioscopy was recorded, looking specifically for the presence of an occludable angle, and location and extent of peripheral anterior synechiae, pigmentation or other abnormalities. An iridotomy was performed in all PACG eyes after controlling the IOP with topical, local and hyperosmotic agents. Medication was then reduced gradually to achieve target IOP with topical medications. In eyes with a glaucoma refractory to maximal tolerated medical therapy, or inability to use medications, a trabeculectomy was performed. All surgeries were done when the IOP was < 21 mm Hg. The interval between iridotomy and surgery was two to six weeks in acute PACG eyes and three months to two years in chronic PACG eyes.

A standard trabeculectomy was performed. A limbus-based conjunctival flap was raised, and a 4 × 4 mm half-thickness scleral flap was dissected. An osteum was made, removing a block of trabecular tissue, approximately 1 × 2 mm. The trabeculectomy tissues were fixed immediately in Karnovsky solution containing 2% paraformaldehyde, 2.5% glutaraldehyde fixative for 12 h at 4^0C. After fixation, the tissues were washed in 0.1 M phosphate buffer saline (pH 7.4) and then post-fixed in 1% osmium tetraoxide for 2 h at 4^0C. The tissues were dehydrated in increasing grades of acetone series (30-100%) and dried in a critical point dryer. The trabeculectomy specimens were mounted on metal stubs to show the intracameral face, and sputter-coated with colloidal gold, 20-nm thick. The specimens were examined under an SEM, Leo 435 VP.

Postmortem eyes of persons with no known ocular pathology, were obtained within an hour of death at our hospital. These eyes underwent a dissection of the limbal area, similar to a trabeculectomy, and the fixation was identical to that for surgical specimens.

With the SEM in secondary electron mode, direct "three-dimensional" images were obtained of the intracameral surface of the trabecular meshwork at magnifications of × 200, × 500, × 1400, × 3000 and × 4000.

Results

Twenty-one glaucomatous trabecular meshwork specimens from 19 adult primary glaucoma patients undergoing trabeculectomy were studied. There were six samples from POAG, 10 chronic PACG and five acute PACG eyes. The patients were aged between 45–69 years, 10 were females and nine males. The baseline uncorrected IOP in POAG eyes ranged from 26–30 mm Hg, in chronic PACG eyes from 28–40 mm Hg and was 52–58 mm Hg in the acute PACG eyes. Five specimens of corneoscleral tissue were obtained immediately postmortem from phakic males aged between 70–80 years, with no known or visible ocular pathology.

Normal trabecular meshwork [Figs. 1a, 2a, 3a]: In all control specimens, the uveal meshwork had 5–12-μm thick, cylindrical beams oriented largely perpendicular to the corneal periphery. The trabeculae were thicker posteriorly. Between the beams many spaces were present, that were 20–30 μm in diameter and larger in the posterior trabecular meshwork. Corneoscleral beams, lying beneath the uveal meshwork, had a smooth, flat shape, and frequently appeared to be oriented parallel to the periphery of the cornea, with smaller spaces. A few melanin granules could be seen on the trabeculae.

Acute angle closure glaucoma eyes: At a magnification of × 200, the intracameral face of all the acute PACG trabecular meshwork specimens appeared to have an irregular surface, in which no landmarks could be appreciated, no trabecular beams were visible and only a few small spaces could be seen [Fig. 1b]. At × 500 and ×1.4 K, in all five specimens, grossly swollen, irregular and crenated trabecular endothelial cells could be seen over the entire surface, with widened intercellular spaces, and basal separation, allowing some cells to be exfoliated or lifted off their bed [Fig. 2b]. There were numerous

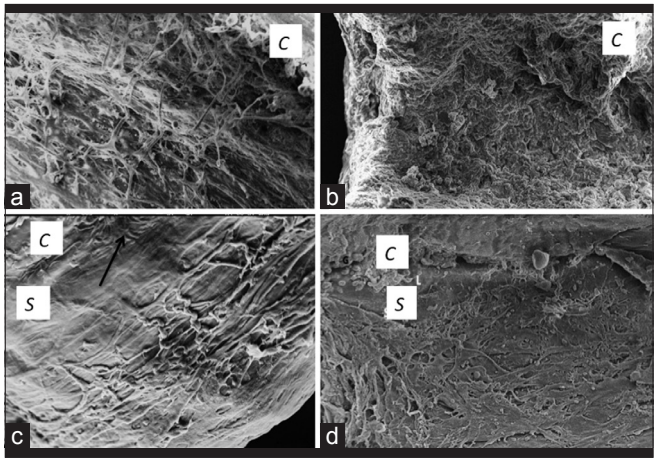

Figure 1: (a) Normal trabecular meshwork × 200; 78-year-old male. There are well-defined thin, rounded uveal beams enclosing many large spaces, running perpendicular to the peripheral cornea, *(C)*. Flatter trabeculae with smaller spaces are seen beneath. (b) Acute PACG eye × 200; 57-year-old female, highest IOP recorded 56 mm Hg. The intracameral face of the trabecular meshwork appeared to have an irregular surface, with no appreciable landmarks. No trabecular beams are visible and only a few small spaces are seen. The peripheral corneal endothelium is also swollen above, *(C)*. (c) Chronic PACG eye × 200; 65-year-old female, highest baseline IOP 40 mm Hg. From above downwards, peripheral corneal guttae, *(C)*, the smooth zone of the trabecular meshwork, *(S)*, and the posterior uveal trabeculae are visible. There are no anterior uveal trabeculae or spaces visible. There is an irregular, fan-shaped configuration of trabeculae converging at the end of the corneal endothelium, (black arrow). (d) POAG eye × 200; 67-year-old male, highest baseline IOP 30 mm Hg. From above downwards, some corneal guttae *(C)*, the smooth zone of the trabecular meshwork *(S)*, and the uveal meshwork are visible. The uveal beams are thinner anteriorly, and become significantly broader posteriorly. The surface shows particulate debris and RBCs

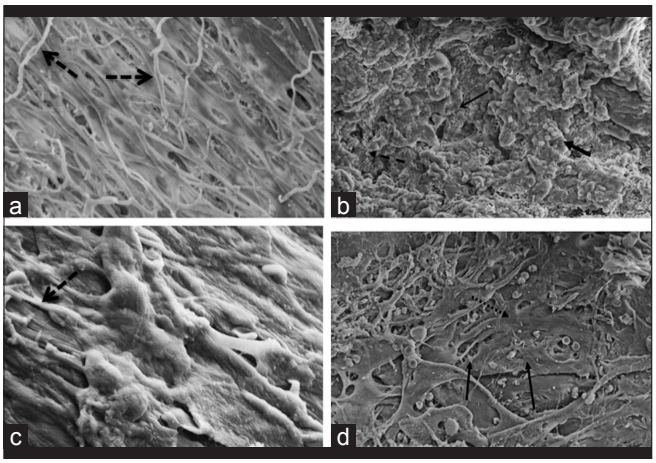

Figure 2: (a) Normal trabecular meshwork, × 500; 72-year-old male. Two to three layers of thinner, more cylindrical, superficial uveal trabeculae are seen, (dashed arrow), overlying flatter corneoscleral trabeculae. The trabeculae are discrete with smooth surfaces, with large, clear spaces between them. A few melanin granules can be seen. (b) Acute PACG eye × 500; 60-year-old female, highest IOP of 52 mm Hg. The trabecular endothelial cells are grossly thickened, irregular and crenated. Some spaces can be seen between the swollen cells, (thin arrow). There are numerous leucocytes on and between the cells (thick arrow). (c) Chronic PACG eye × 500; 62-year-old male, baseline IOP of 38 mm Hg. There are cylindrical trabeculae seen to the left, (dashed arrow). Posterior trabeculae to the right are flat, widened, irregularly branching, and nodular, with spaces seen between them. (d) POAG eye × 500; 45-year-old male, highest IOP 28 mm Hg. A few uveal trabeculae can be seen, (dashed arrow), with most being seen to be covered/ sheathed by a homogeneous material, (full arrows), which appears to bridge intertrabecular spaces, as well. Numerous melanin granules, together with fibrillar debris are present on the surface

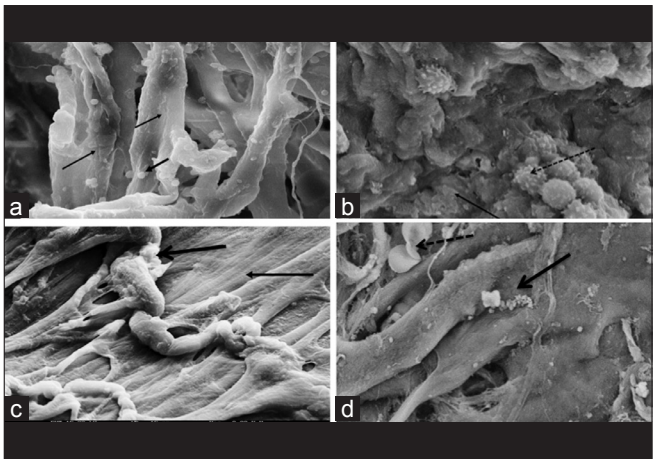

Figure 3: (a) Normal trabecular meshwork, × 3.0K. Each cylindrical uveal trabecular beam is distinct, with generally smooth endothelial surfaces, and some cellular borders visible (thin arrows). Large intertrabecular spaces are present. Melanin granules, (thick arrow), can be seen on the cells and within the spaces. (b) Acute PACG eye × 3.0K; 65-year-old female, highest IOP recorded 54 mm Hg. The swollen endothelial cells show an irregular surface with numerous macrophages, (dashed arrow), and an accumulation of pigment granules in an amorphous deposit (full arrow). (c) Chronic PACG eye × 3.0K; 57-year-old male, baseline IOP of 36 mm Hg. Few widened uveal trabeculae with a knotted appearance are visible. The knotted appearance appears to be due to the presence of nuclei of the endothelial cells in some places, and in others due to the localized accumulation of amorphous material (thick arrow). Other trabecular beams can be seen within a homogenous deposit (thin arrow), with few spaces or corneoscleral trabeculae visible. (d) POAG eye × 3.0K; 67-year-old male, highest baseline IOP 28 mmHg. There are flat and widened uveal trabeculae, beneath which corneoscleral trabeculae and spaces are seen. Fewer spaces are visible cf control eyes. Fused trabeculae can be seen (thick arrow). RBC (thin arrow), melanin granules and debris are seen on the surface

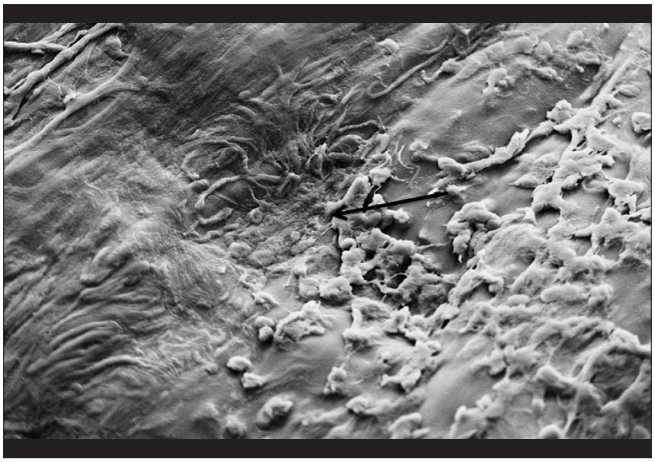

Figure 4: CPACG eye × 1.4K; 62-year-old male, baseline IOP of 38 mm Hg; showing converging and fusion of anterior uveal trabeculae where corneal endothelial cells (arrow) can be seen to end. There are no intertrabecular spaces seen around. This was at the site of a peripheral anterior synechia on gonioscopy

leucocytes, activated macrophages, and amorphous debris, adhering to and between the trabecular cells in all samples. No trabeculae could be identified superficially, and there were none visible in the few spaces present [Fig. 3b]. At × 1.4K and 3.0 K, widened intercellular junctions could be seen between the swollen cells. Some degenerated cells could be seen. Melanin granules and fibrillar material were present around the cells [Fig. 3b].

Chronic PACG eyes: At a magnification of × 200, seven out of 10 trabeculectomy specimens had some posterior uveal trabeculae and spaces visible but very few anteriorly. In three specimens, fan-shaped configurations of irregular trabeculae were seen converging towards the cornea [Fig. 1c], corresponding to areas of peripheral anterior synechia seen preoperatively on gonioscopy [Fig. 4]. The intracameral surface showed thick, knotted, ropy, perpendicularly oriented uveal trabecular beams in eight out of 10 specimens. The anterior trabecular meshwork had a homogenous, sheet-like deposit between and upon strap-like trabeculae, which could be seen in relief, obliterating all orifices. Posteriorly, the deposit could be seen between and beneath the few visible trabecular beams in all specimens. At a magnification of × 500 the deposits were more extensive anteriorly, where no uveal or corneoscleral trabeculae or spaces could be seen [Fig. 2c]. At a magnification of × 1.4K, numerous trabeculae were seen to be embedded in the homogenous layer [Fig. 3c]. Under × 3.0K magnification, the surface of the trabecular endothelium was seen to be irregular, and studded with pigment granules.

The knotted appearance was seen to be due to the presence of nuclei of the endothelial cells in some places, and in others due to the localized accumulation of amorphous material. The deeper corneoscleral trabeculae and orifices were also enmeshed in the homogenous material. A few red blood cells and leukocytes were seen.

Primary open angle glaucoma: In POAG eyes, four trabecular specimens at a magnification of × 200 showed from anterior to posterior [Fig. 1d], some corneal guttae, the smooth zone of the trabecular meshwork and the uveal meshwork. Uveal trabecular beams were wider posteriorly, but were significantly broader than control eyes at all locations. There were more intertrabecular spaces seen anteriorly, and only a few posteriorly. The surface had a moderate amount of particulate debris and RBCs. At a magnification of × 500, the anterior uveal beams were seen to be about 3-6-μm wide, strap-like, and appeared to branch irregularly [Fig. 2d]. The posterior beams were 6–50-μm wide, irregularly arranged, with some beams appearing to merge together. In five out of six specimens, there was a homogenous deposit bridging some of the beams. Numerous trabecular beams were visible in relief within this membranous formation. Looking through the small, oval intertrabecular spaces between anterior uveal trabeculae, a number of underlying trabecular layers and spaces were visualized. Posteriorly, the uveal trabeculae left fewer but larger spaces, beneath which some corneoscleral trabeculae and smaller spaces were visualized. With a higher magnification of × 1.4K, numerous trabeculae were seen to be sheathed by the homogeneous material, which bridged intertrabecular spaces, forming a sheet [Fig. 3d]. The surfaces of some trabeculae were irregular. Through the intertrabecular spaces of the uveal trabeculae, thinner corneoscleral trabeculae and spaces were visible. There were some holes seen in the film-like structure. A few red blood cells, macrophages, melanin granules approximately 1 μm in size, together with irregular amorphous and fibrillar debris were seen on the surface and in the trabecular orifices of all specimens. Trabecular endothelial cells' borders could not be identified. At a magnification of × 3.0K, a fibrillar appearance was seen at the edges of the sheet-like structure, deposited between trabeculae.

Discussion

There is a paucity of literature on trabecular meshwork pathology in primary adult glaucomas, and even less on changes seen in eyes having chronic PACG or acute PACG. This is the first study to compare the SEM appearance of the intracameral trabecular surface in acute PACG, chronic PACG and POAG eyes.

There have been a number of SEM studies on normal trabecular meshwork.[6-9] The uveal meshwork has been reported to have 5-12-μm thick, cylindrical beams with a rough surface, running from the iris root, perpendicular to and up to the corneal periphery. Between the beams many spaces were present, that were 20-30 μm in diameter, with a highly permeable aspect. The corneoscleral beams, lying beneath the uveal meshwork, had a smooth, flat shape, and were oriented parallel to the periphery of the cornea, flanking smaller spaces.

All acute PACG eyes in our study showed the presence of swollen cells with basal separation, obscuring trabecular beams, and almost obliterating intertrabecular spaces. There were also many leucocytes and some activated macrophages seen in these eyes. A previous histopathological study by Sihota *et al*., had also shown generalized edema of trabecular beams, desquamated endothelial cells seen between the trabecular beams, pigment dispersion and activated, phagocytic endothelial cells.[3] In an experimental study Grierson and Lee[10] found that raising IOP to 50 mm Hg for 1 h in monkey eyes led to a pronounced distension of the endothelial and corneoscleral layers of the meshwork. The posterior meshwork contained isolated cellular elements and fragmented and disorganized extracellular material. The endothelial cells contained an abnormally large number of lysosomes, lysosomal complexes and lipid vesicles. In another experimental study in monkeys, Svedberg[11] maintained an IOP of 34-44 mm Hg for a number of hours, and found trabecular disruption and total detachment of complete trabecular endothelial cells in such eyes. It appears that the endothelial cell edema seen in acute PACG eyes could be the result of the high IOP seen in such eyes, and could itself further obstruct aqueous outflow, leading to even higher intraocular pressures, which do not resolve with maximal antiglaucoma therapy. Therapy should therefore include anti-inflammatory medications topically and systemically with IOP-lowering medications, to control the inflammatory cells and reduce trabecular edema.

In this study, chronic PACG eyes showed an expansive deposition of homogenous material covering preexisting trabeculae and their orifices completely in the anterior trabecular meshwork. The posterior uveal meshwork beams were rounded, thickened and knotted, with irregular branching and orientation. A fan-shaped convergence of anterior trabecular beams could be seen in areas having peripheral anterior synechiae on gonioscopy, a rearrangement possibly secondary to endothelial cell loss/damage by iridotrabecular adhesions. These trabecular changes were seen to be structural, and irreversible. Transmission electron microscopic studies of chronic PACG have shown the loss of trabecular architecture and its regular arrangement, with fewer and narrower trabecular spaces, and fusion of trabecular beams in areas.[3] Numerous electron-dense bodies were present in the trabecular tissues, both within the trabecular beams and in the extracellular spaces, which had a banded fibrillar structure. An overall loss of endothelial cells was noted, the remaining cells being crowded together and polymorphic. Tripathi described an eye with closed angle glaucoma in absolute stage, reporting widespread disorganization and degeneration of the trabecular endothelium and increased phagocytosis of pigment.[4] Lee found the presence of inflammatory cells, usually lymphocytes in the trabecular spaces of angle closure glaucoma eyes unresponsive to therapy.[2] He also noted numerous melanin granules in the trabecular endothelial cells. Changes on SEM are seen both in areas of peripheral anterior synechiae, as also without, suggesting that an iridotomy or cataract surgery in such eyes may prevent further attacks of angle closure, but will not reverse the structural obstruction to aqueous outflow.

In our study, POAG eyes had sparse uveal trabecular beams that appeared flat, irregularly arranged and some seemed embedded in a sheet-like material, especially posteriorly. Under higher magnification, it was possible to see preexisting trabecular beams and the orifices partially or totally enmeshed in the material seen. In other areas the individual uveal beams were still visible, and their intertrabecular spaces revealed

thinner corneoscleral trabeculae and spaces beneath. Chaudhry *et al*., described a loss of trabecular beam outlines due to a homogenous deposit overlying and partially obstructing the trabeculae, in POAG eyes.[9] They found the surface quality varied from smooth to granular or nodular. This was refuted by Quigley *et al*., who did not find any deposit obstructing the pores of the POAG eyes studied by them.[12] Potau *et al*., reported the presence of a dense, homogenous, continuous material covering the intracameral surface, partially or totally obstructing the orifices, in 50% of POAG specimens seen. In some cases they were able to see cell-like structures superficial to this material, which they thought were migrating trabecular endothelial cells. They ruled out the possibility of the deposits being secondary to processing of the tissue.[6] Segawa postulated that a polysaccharide protein complex may be produced by the endothelial cells of the trabecular meshwork.[13] In POAG eyes, we were unable to identify clearly the trabecular endothelial cells or their margins, as previously reported by Sampaolesi and Argento.

The homogenous layer seen in both POAG and chronic PACG could possibly be a SEM correlate of electron-dense material described by transmission EM in both POAG and chronic PACG eyes. Migration of endothelial cells to compensate for lost cells or migration during phagocytosis could lead to the deposition by these cells, of the homogenous material present in such eyes. The total or partial enmeshing of preexisting trabecular beams and intertrabecular spaces, appears akin to the process of fossilization, wherein more resistant parts i.e. the trabecular beams, appear preserved within layers deposited over time, whereas friable tissue such as the endothelium is lost.

Both POAG and chronic PACG eyes seen by us showed irregular branching and fusing together of trabeculae to form broadened trabecular beams. Fine *et al*., also noted trabecular fusion in the posterior meshwork resulting in the loss of drainage pathways.[14] This was more dramatic in POAG eyes as compared to normals in their study, and was found in areas where there was a marked depletion of endothelial cells. Alvarado *et al*., described fewer endothelial cells in the trabecular meshwork of POAG eyes than controls.[5] Teng *et al*., suggested that a reduction in aqueous outflow affects the metabolic support to trabecular endothelial cells contributing to degeneration or loss of these cells.[15] It is debatable whether a raised IOP causes a loss of endothelial cells, trabecular fusion and decreased aqueous outflow, or whether a decreased aqueous outflow leads to a disturbance of the metabolism of endothelial cells, and the changes seen on histopathology.

The main limitation of our study was the small size of the surgical tissue available, and the difficulty in orienting the specimens with the trabecular surface uppermost, as also their anteroposterior orientation. We had to exclude four specimens, as the trabecular meshwork could not be visualized after mounting. In our study there was one patient aged 45, and two aged 57 years, the rest were in their 60s, as compared to the control patients who were older, as they were postmortem specimens. Alvarado *et al*., reported a loss of 0.0074 nuclei/ unit tissue area/ year, which in a decade or so would be negligible. The trabecular structure in control eyes was, as described, with well-defined trabeculae, many intertrabecular spaces and endothelial cells that were well visualized cf glaucomatous eyes. Another limitation was our inability to view the posterior trabecular meshwork in a few eyes, especially acute PACG, so that although we attempted to take the surgical specimens away from areas of peripheral anterior synechiae (PAS), evidence of these was visible in some photographs.

This study was the first to look at SEM in PACG eyes, and has provided evidence of fewer intertrabecular spaces in chronic PACG, that could account for the higher IOP seen in chronic PACG eyes as compared to POAG. The findings of swollen endothelial cells blocking the trabecular spaces in acute PACG eyes go some way towards explaining the initial very high IOP, that over time, resolves in a considerable number of patients. There was a wide array of pathological alterations seen on the intracameral surface of the trabecular meshwork in PACG eyes as compared to normal and POAG eyes. The SEM changes described, could help in the rational therapy of PACG. The generalized edema of the trabecular endothelium and presence of inflammatory cells in acute PACG eyes associated with an acute and marked rise of IOP would probably respond better to anti-inflammatory therapy added to maximal glaucoma therapy. The gradual, chronic rise of IOP in chronic PACG is probably due to progressive fusion of adjacent trabeculae, leading to widened and irregular trabeculae, as well as the homogenous material deposited on and between the trabeculae. Additionally, areas of peripheral anterior synechiae showed structural alterations of trabeculae and narrowing of intertrabecular spaces.

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