

Pathology and immunohistochemistry of capsular bag in spontaneously late dislocated capsular bag-intraocular lens complex

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Purpose: Our study aims to evaluate the morphology, histopathology, and immunohistochemistry of the spontaneously late dislocated capsular bag-intraocular lens (CB-IOL) complex. Various etiologies and possible pathogenesis of the event are also discussed. **Methods:** This was a tertiary-care setting and retrospective observational case series. The surgically explanted intact specimens of spontaneously late dislocated CB-IOL complex were studied. The demographics, duration of pseudophakia, IOL design/material, and specimen measurements were noted. Fresh specimens were photographed, and computer software was used for measurements. After processing, a detailed microscopic examination was carried out for three different sections of each specimen with hematoxylin and eosin (H and E), Masson's-trichrome, and immunohistochemistry stain for vimentin. The Mann-Whitney U-test was used for the statistical analysis. **Results:** Of 12 specimens, the mean CB and capsulorhexis opening size were 8.32 ± 0.8 mm and 3.62 ± 0.61 mm, respectively. The average CB-IOL complex size of our study was significantly lower than the studies reported in the literature ($P \leq 0.001$). All ($n = 12$, 100%) were acrylic IOLs with 11 (91.67%) having single-piece design. All specimens on H and E stain showed extensive subepithelial fibrosis while Masson's trichrome staining showed that none had any pseudoexfoliation material. The circumferential sphincter-like fibrous tissue arrangement was seen in all specimens. Immunohistochemical expression of vimentin suggested the mesenchymal metaplasia of epithelial A-cells. **Conclusion:** Significant fibrotic contraction of the CB and phimosis of capsulorhexis may cause a progressive zonular tear. This is probably the most important etiology of spontaneous late dislocation of the CB-IOL complex.

Key words: Late capsular bag-intraocular lens complex dislocation, lens capsule contraction, lens capsule immunohistochemistry, pseudoexfoliation syndrome

Spontaneous anterior or posterior dislocation of capsular bag-intraocular lens (CB-IOL) complex is an uncommon but serious complication after cataract surgery. It is defined as early or late depending on the duration of cataract surgery with IOL placement. Any dislocation occurring within 3 months of cataract surgery is defined as "early" and after 3 months as "late" CB-IOL complex dislocation.^[1] Inappropriate IOL fixation secondary to posterior capsular rupture or zonular dialysis may predispose and lead to early CB-IOL complex dislocations while the etiopathogenesis of spontaneous late CB-IOL complex dislocations is not established.^[1,2]

The reported incidence of spontaneously late CB-IOL complex dislocation is 0.05%–3.0%.^[1] Severe decentration or dislocation is the most common indication for surgical explantation of an acrylic foldable IOL.^[2] Average time interval between primary surgery and spontaneous late in-the-bag IOL dislocation is 8-15 years and its incidence appear to be steadily rising globally, particularly after the invention and global practice of continuous curvilinear capsulorhexis (CCC).^[1-5] Various studies have found pseudoexfoliation (PEX), high myopia, uveitis, previous

attack of acute angle closure glaucoma, and vitreous surgery to be the associated risk factors for late CB-IOL complex dislocation.^[1-5] Aging has been mentioned as an additional/adjunctive risk factor which questions the overall significance of the above mentioned.^[6]

Krèpštè *et al.* found that the majority of spontaneous late CB-IOL complex belonged to "in-the-bag" variety as only 12.1% were "out-of-bag" CB-IOL dislocated complexes.^[7] In literature, capsule contraction and phimosis of capsulorhexis opening have been hypothesized for late CB-IOL complex dislocation.^[1-5,9] A few authors also believed that progressive zonular weakness is the most common causative factor for CB-IOL complex dislocation.^[5,7]

Hence, we planned the current study evaluating anatomical, histopathological, and immunohistochemical features of surgically explanted specimens of spontaneous late CB-IOL complex dislocation.

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Cite this article as: Bhattacharjee H, Bhattacharjee K, Das D, Singh M, Sukumar P, Misra DK. Pathology and immunohistochemistry of capsular bag in spontaneously late dislocated capsular bag-intraocular lens complex. Indian J Ophthalmol 2017;65:949-54.

Access this article online

Website:

www.ijo.in

DOI:

10.4103/ijo.IJO_790_16

Quick Response Code:



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Manuscript received: 12.10.16; Revision accepted: 31.07.17

Methods

This retrospective, observational study was carried out over the consecutive surgically retrieved specimens of spontaneous late in-the-bag CB-IOL complex dislocations from 2010 to 2015. Our study was approved by the Institutional Review Board. Demography, clinical history, and histopathological details were recorded from the case file of each patient.

All specimens were surgically explanted through a standard 3-port pars plana vitrectomy (PPV) and the CB-IOL complex was delivered through a clear corneal incision. Then, a prolene suture-assisted scleral fixation of nonfoldable poly methyl methacrylate IOL (6.5 mm optic diameter) was implanted. All the retrieved specimens were microscopically examined instantly by an ocular pathologist (DD). Specimens with ruptured CBs were excluded from the study. IOL design and material was noted and confirmed using the operative records. The actual overall IOL length and optic diameter size were recorded from the IOL identification stickers pasted over files.

Procedure

At room temperature, the wet specimens were placed over a glass slide, and digital microphotographs were taken using a microscope-fitted Kodak M200 (Rochester, New York, USA) camera. After that, specimens were immersed in 10% neutral buffer formalin for pathological evaluation. The specimens were dehydrated, processed, and embedded in paraffin blocks. Multiple 3 μm -thick sections were cut and stained with hematoxylin and eosin (H and E), Masson's trichrome, and immunohistochemistry staining for vimentin.

Axioskop-40 (Carl Zeiss, Göttingen, Germany) compound microscope with AxioCam MRc 5 (Carl Zeiss, Göttingen, Germany) camera was used for the examination and photomicrographic documentation. Pathological changes of the CB, anterior capsule and the deposits of an amorphous material over the outer surface of anterior lens capsule were observed. The clinical diagnosis was masked from the pathologist during the whole process. Diffraction microscopy was done without staining the specimen.

Staining methodology

Three different sections from each CB were stained with three different stains: H and E (histopathology), Masson's trichrome (PEX identification), and vimentin (immunohistochemistry).

Photomicrograph measurement

Clinical photographs were evaluated by computer-based online Image J software (version J2, SciJava) by National Institutes of Health, US. The diameters of CB and capsulorhexis were measured using the first photographs taken immediately after explantation. Size, morphology, and IOL morphology was also noted. Standard IOL optic (6mm diameter) image was used to calibrate the measurement. In ambiguous images, three measurements were taken, and the average was used.

The Mann-Whitney U-test was used for statistical comparison between the sizes of retrieved CB-IOL complex specimens from our study and those of existing literature.

Results

Twelve explanted specimens (from 12 eyes) of spontaneous late in-the-bag CB-IOL complex dislocation were included in our study. There were ten males and two females having a mean age of 70 ± 7.1 years (median, 70 years) at the time of explantation surgery.

All PPV surgeries were performed by a single surgeon (HB) at a mean interval of 11.6 ± 2.9 years (median, 10 years) after primary cataract surgery + IOL implantation. The precataract surgery medical records showed inadequate pupil dilatation ($n = 4$), high myopia ($n = 3$), primary open angle glaucoma (POAG) ($n = 1$), blunt ocular trauma ($n = 1$), and intermediate uveitis ($n = 1$). None of the patients showed features of PEX before cataract surgery. Phacoemulsification with a foldable IOL implantation was the primary cataract surgical procedure performed in all patients. Trypan blue dye was not used in any of the cases during cataract surgery. The glaucoma patient was being managed with topical, intraocular pressure-lowering agents. Eleven were foldable single piece (FSP) acrylic lenses while one specimen contained foldable 3-piece acrylic IOL. One specimen containing FSP lens also had a capsular tension ring inside an intact CB (though it ruptured while handling with fine forceps during photography, [Fig. 1]).

Each explanted specimen included an intact CB containing hydrophobic acrylic foldable IOL. The mean CB size was 8.32 ± 0.8 mm (median, 8.33 mm) while the mean capsulorhexis opening measured 3.62 ± 0.61 mm (median, 3.76 mm), suggesting the CB contraction and phimosis of capsulorhexis opening in all. Variable grades of Soemmering's ring were present in all [Figs. 2 and 3]. Gross anterior capsular opacity along with capsular wrinkles was prominently observed in each specimen, especially at contact site between the anterior capsule and IOL optic as well as the haptic [Fig. 4]. The haptics of IOL

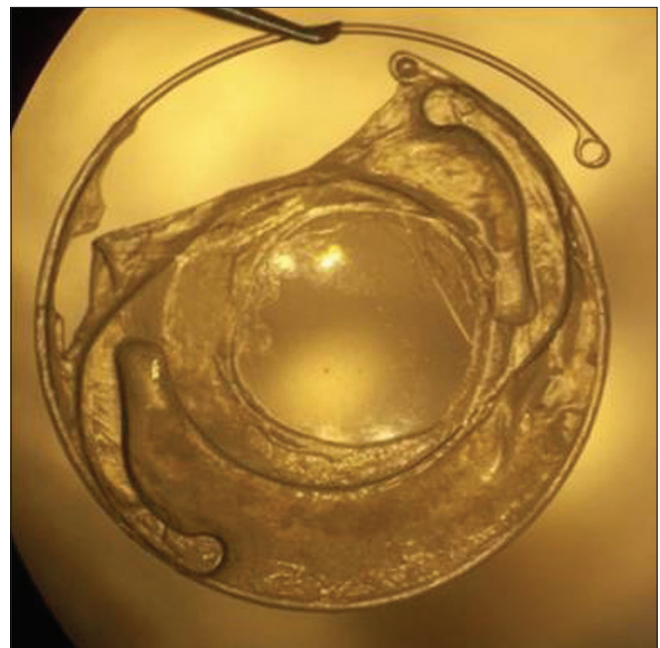


Figure 1: Explanted specimen showing capsular tension ring and single piece intraocular lens in-the-bag. Capsulorhexis phimosis is also observed

Table 1: Clinical data for 12 explanted samples of dislocated capsular bag-intraocular lens complex

Age	Sex	Duration of pseudophakia (years)	Grade of ring of soemmering	Capsulorhexis opening size (mm)	Size of capsule bag (mm)	IOL design and material
68	Male	9	Mild	4.02	7.93	FSP
71	Female	14	Severe	3.01	8.22	FSP
69	Male	9.5	Moderate	4.78	8.17	FSP
75	Male	15	Moderate	2.58	8.53	FSP
84	Female	17	Severe	3.06	8.19	FSP + CTR
70	Male	10	Severe	3.8	9.29	FSP
65	Male	10	Moderate	2.96	8.5	FSP
63	Male	9	Mild	3.6	7.5	FSP
78	Male	13	Severe	3.76	8.33	FSP
61	Male	8	Mild	3.9	8.91	F3P
63	Male	9	Moderate	3.91	6.58	FSP
73	Male	13	Severe	4.04	9.68	FSP

FSP: Foldable single-piece, F3P: Foldable 3-piece, CTR: Capsule tension ring, IOL: Intraocular lens



Figure 2: Gross photograph of an explanted capsular bag-intraocular lens complex showing three-piece hydrophobic acrylic intraocular lens. A well-formed doughnut-shaped Soemmering ring, a phimosed oval-shaped capsulorhexis, and shrunken capsular bag is noticeable in the specimen

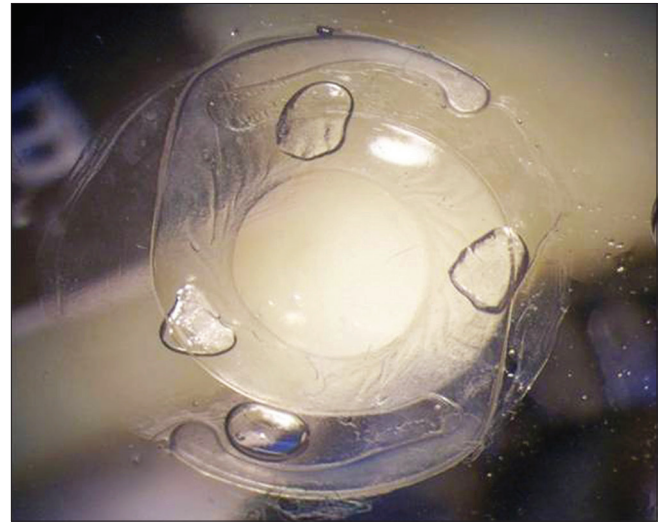


Figure 3: Gross picture showing a single-piece hydrophobic acrylic intraocular lens. Specimen shows fibrosis of anterior capsule (at the point of contact between anterior capsule and IOL optics as well as haptics), phimosis of capsulorhexis and shrunken capsular bag. The intraocular lens haptics appears compressed toward optic. Size of intraocular lens, capsular bag, and capsulorhexis is 9.29 mm, 9.29 mm, and 3.8 mm, respectively

was found to be compressed in all the specimens, as suggested by the reduced overall length of IOL [Table 1]. Anterior flexion of IOL haptics was also observed in two specimens.

Histopathological analysis of the explanted specimens was performed by single ocular pathologist (DD). Each specimen was sliced in multiple 3µm thick sections. Microscopically, a ring pattern of fibrosis surrounding the capsulorhexis opening was seen under the anterior capsular epithelium [Fig. 5]. H and E staining showed lens capsular epithelium (A-cells), extracellular matrix accumulation, presence of elongated fibroblasts, and presumed equatorial E-cells [Fig. 4]. Masson's trichrome stain did not reveal any deposition of amorphous substance on the external surface of the anterior capsule in an iron-filling pattern [Fig. 4].

Immunohistochemical studies detected fibrous collagen type and cellular fibronectin. Presumed lens epithelial cells (LECs) in

the middle of extracellular matrix-stained positive for vimentin and alpha smooth muscle actin.^[3] Diffraction microscopy showed migrating E-cells, torn zonules, and compression fold of the IOL haptics [Fig. 6].

Discussion

Exact etiopathogenesis of spontaneous late CB-IOL complex dislocation is not known. This delayed and serious postcataract surgery complication has been correlated with progressive weakness of zonular fibers, capsule bag contraction, and phimosis of capsulorhexis opening.^[7,9] Other conditions leading to zonular weakness are surgical stress, PEX, high myopia, glaucoma, previous vitreoretinal surgery, connective tissue disorders, retinitis pigmentosa, etc.^[3-12] These conditions may cause diffuse and progressive weakness of the ciliary zonules,

leading to instability of the CB containing the IOL, which may get dislocated by the smallest amount of pressure or gravity. On detecting CB fibrosis and contraction, radial cuts with Nd:YAG capsulotomy are considered to have a preventive role in CB-IOL dislocation.^[13]

CCC, cataract extraction, and IOL implantation inadvertently results in changed anatomy of CB, zonules, and ciliary body. In combination, all these changes alter the normal anatomical and physiological relationship, geometry, and dynamics of the lens capsule.^[8-10,14] The radial diameter of empty CB enlarges from average normal 9.5 mm to 10.8 mm (approximately), secondary to stretching of the lens equator by elastic zonules. This causes apposition of CB fornix and ciliary body along with the posterior shift of lens equator.^[8,17] The diameter of the empty CB is larger than that of the anterior ciliary ring. Ultrasound biomicroscopy and magnetic resonance imaging studies have shown discordant results demonstrating reduction and no

change in ciliary ring diameter after IOL implantation.^[10-12] Moreover, secondary to the flattening of the posterior capsule, there is anterior vertical shift and posterior repositioning of the ciliary processes. The angle between anterior and posterior zonular limbs also gets reduced to 10°–15° from the normal 45°.^[11]

In our study, we found a reduction of the capsule bag size (8.32 ± 0.8 mm) and phimosis of capsulorhexis opening (3.62 ± 0.61 mm) which might have occurred secondary to gross contractions by intracapsular fibrosis [Table 2]. The Mann–Whitney U-test suggested that the average specimen size reported by our study is significantly lower than the previous studies reported in literature ($P < 0.001$) [Table 3].

Typical concentric fibrotic bands were seen on the interior of CCC which might have led to the contraction of anterior capsule leading to stretch on ciliary zonules. This fibrotic activity is secondary to the metaplastic changes in the residual LECs inside the CB which inherit the fibro-contractile activity. It might also explain the folds over anterior capsule and the compression of IOL haptics causing a reduction in overall length of IOL. In two cases, anterior flexion of the haptics was observed which again goes in favor of capsular fibrosis. Literature supports the phimosis of capsulorhexis opening as a significant finding in the late dislocation of CB-IOL complex.^[3,5-8]

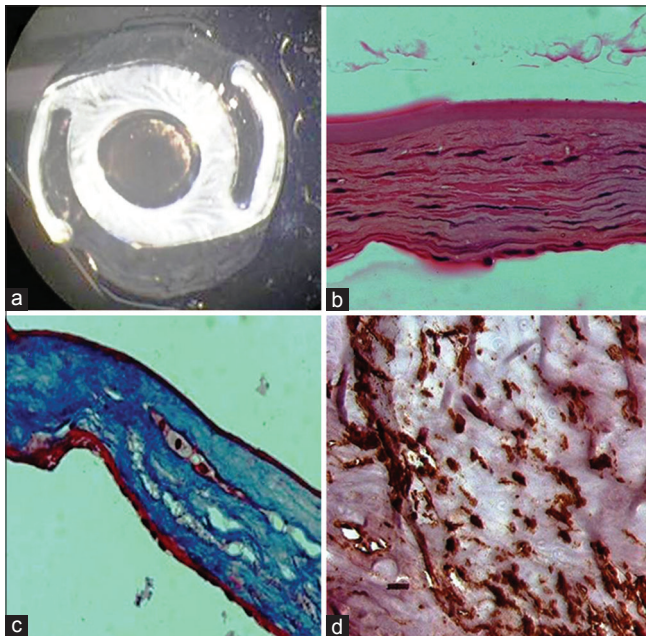


Figure 4: (a) A light photograph showing folds in the anterior capsule and a phimosed capsulorhexis. Anterior capsular opacity seen universally at the contact site of intraocular lens and anterior capsule. (b) Histopathology of capsule with hematoxylin and eosin stain shows an intact capsular epithelium, accumulation of extracellular matrix, and subepithelial elongated fibroblast cell. (c) Masson's trichrome stained-negative for pseudoexfoliation material. (d) Immunohistochemistry shows expression of vimentin (brown stain)

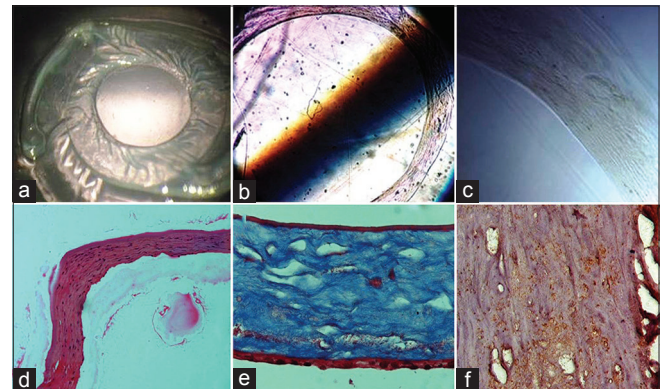


Figure 5: (a) Severe capsular and continuous curvilinear capsulorhexis phimosis with anterior capsular wrinkles. (b and c) Magnified view shows sphincter-like arrangement of fibrous tissue around continuous curvilinear capsulorhexis. (d) Hematoxylin and eosin stain shows dense subcapsular fibrous tissue. (e) Masson's trichrome staining reveals basement membrane with nuclei in the lens epithelial cells (dark granules). Dense subcapsular fibrosis due to LEC metaplasia is also seen. (f) Immunohistochemistry detected the expression of Vimentin (brown)

Table 2: Comparison of the size of the capsular bag by various authors

Author	Year	Capsule size (mm)	Specimen retrieved from	Status of capsular bag during measurement
Richburg and Sun ^[14]	1983	10.8±1.4	Cadaver	Empty bag
Galand <i>et al.</i> ^[15]	1984	10.32±0.42	Cadaver	Empty bag
Assia and Apple ^[16]	1992	10.5	Cadaver	Both empty bag and crystalline lens evaluated <i>in-situ</i> through uveoscleral window
Vasavada and Singh ^[17]	1998	10.38±0.35	Cadaver	Empty bag
Modesti <i>et al.</i> ^[10]	2011	9.95±0.80	<i>In vivo</i>	<i>In vivo</i>
Present study	2016	8.32±0.82	Explanted specimen	IOL in capsular bag

IOL: Intraocular lens

Table 3: Statistical comparison of mean capsular bag size between the present study and various studies in literature

Studies compared	Mean capsular bag size of present study (mm)	Mean capsular bag size of the study in literature (mm)	P value of Mann-Whitney U-test of medians	Remarks
Present study versus Richburg and Sun ^[14]	8.32±0.82	10.8±1.4	<0.001	Significant at 1%
Present study versus Galand <i>et al.</i> ^[15]	8.32±0.82	10.32±0.42	<0.001	Significant at 1%
Present study versus* Assia and Apple ^[16]	8.32±0.82	10.5	-	Significant at 1%
Present study versus* Vasavada and Singh ^[17]	8.32±0.82	10.38±0.35	-	Significant at 1%
Present study versus* Modesti <i>et al.</i> ^[10]	8.32±0.82	9.95±0.80	-	Significant at 1%

*Mann-whitney U-test could not be done due to non-availability of the data in the published article but P value of the *t*-test of means in between present study and later three studies in the table 3 (15,16 and 9) showed statistically significance

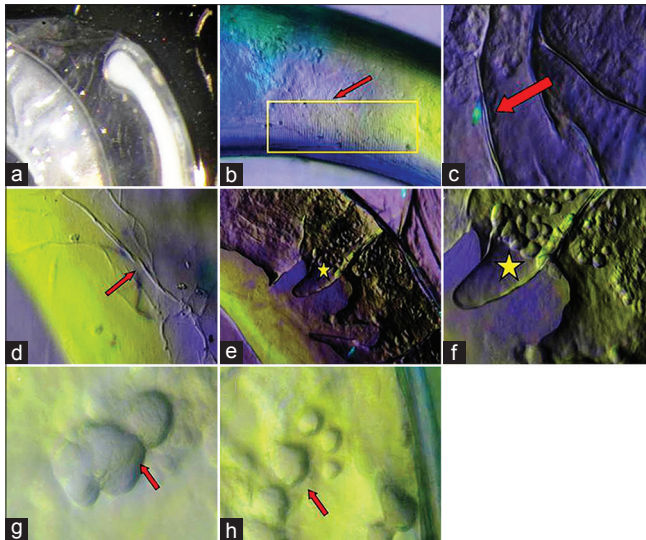


Figure 6: (a) Contraction of capsular bag leading to haptic-optic approximation. (b) Diffraction microscopy shows vertical compression folds (yellow box) and torn zonules. (c and d) Fibrous tissue strands seen growing such as cords over the haptics (arrows). Migrating sheets of regenerative cortical E-cells ([e and f] asterix) and bladder cells ([g and h] arrow)

Microscopically, the fibrous tissues were arranged in a concentric circular manner surrounding the capsulorhexis opening, which may cause a purse-string or tonically contracted sphincter-like effect [Fig. 5]. The other parts of capsule show irregular type of thicker fibrotic tissue deposit mainly in periphery and IOL optic-haptic junction. Immunohistochemical studies of all specimens showed expression of vimentin, suggestive of mesenchymal transformation of the A-cells of the lens capsule. This A-cell metaplasia occurred predominantly at the site of contact between IOL and the anterior capsule. This indicates that the IOL material may play a role in capsular fibrosis.

Biointerface multiparametric study as well as other investigations have demonstrated that the acrylic material of IOL is associated with increased cellular activity inside the CB when IOL is *in situ*.^[18,19] Influence of IOL features and material has a significant effect on the fibrotic activity of lens capsule, proliferation, and migration of LECs. This can cause contraction and opacification of the anterior as well as posterior capsule. Experimentally, migration of LECs can be prevented by keeping the bag empty and inflated, i.e., without IOL.^[19-21]

Intracapsular fibrosis and gross capsular contraction were the single common pathological finding in all our specimens. Other mentioned clinical features (resistant pupil, high myopia, POAG, ocular trauma, and intermediate uveitis) which may contribute to zonular weakening and dislocation were coexisting. However, our study found no pseudoexfoliative material in any of our explanted specimens making it a unique finding. The authors believe that a dynamic, strong, and constant centripetal force directed anteriorly toward the center of the capsulorhexis caused by uncontrolled fibrosis in the CB, and capsule contraction is the main causative factor for progressive tearing of the suspensory ligaments leading to spontaneous late in-the-bag CB-IOL complex dislocation in our cases. Similar microscopic features have been observed by other researchers as well.^[17]

Although our study has limitations such as small sample size and no data of intraoperative capsulorhexis size, we have tried to scientifically extrapolate the microscopic signs to this clinical situation. We conclude that fibrosis and capsular contraction is a natural, *in vivo* consequence of cataract extraction and IOL implantation. However, if the capsular contraction is excessive, as found in the present study, it may be a significant factor causing late in-the-bag IOL dislocation. Further, research is needed to establish the cause and effect relationship. Adequate CCC and an impeccable cleaning of lens capsule from all the residual epithelial cells are of importance and might prevent this delayed complication. Fibrous activity in the capsule bag may be modifiable and preventable and so is the late in-the-bag IOL dislocation. Adequate and timely surgical management of this complex situation may provide good clinical outcomes.

Conclusion

Fibrous contraction of the capsule and immunohistochemical expression of Vimentin in late dislocated specimens of CB-IOL complex found in the present study suggests mesenchymal transmission of LECs and it might be related to bioincompatibility of the IOL material in some individual. Because all cases of pseudo exfoliation do not land up with later CB-IOL dislocation. This facts indicates multiple and individual factors are responsible for this late devastating complication.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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