

# Electron microscopic investigation of anterior lens capsule in an individual with true exfoliation

*Xing-Chao Shentu<sup>1</sup>, Ya-Nan Zhu<sup>1</sup>, Ying-Hui Gao<sup>1</sup>, Su-Juan Zhao<sup>1</sup>, Ye-Lei Tang<sup>2</sup>*

**Foundation item:** Zhejiang Key Innovation Team Project of China (No. 2009R50039)

<sup>1</sup>Eye Center, the 2<sup>nd</sup> Affiliated Hospital of Medical College, Zhejiang University, Hangzhou 310009, Zhejiang Province, China

<sup>2</sup>Department of Neurology, the 2<sup>nd</sup> Affiliated Hospital of Medical College, Zhejiang University, Hangzhou 310009, Zhejiang Province, China

**Correspondence to:** Ye-Lei Tang. Department of Neurology, the 2<sup>nd</sup> Affiliated Hospital of Medical College, Zhejiang University, #88, Jiefang Road, Hangzhou 310009, Zhejiang Province, China. stxc20030304@sina.com

Received: 2013-04-16 Accepted: 2013-07-02

## Abstract

• **AIM:** To determine the changes which occur in the anterior capsule in true exfoliation which is a very rare condition.

• **METHODS:** The anterior capsule from a 93 year-old patient and 6 other patients with age-related cataract during capsulorhexis was examined *via* transmission electron microscopy (TEM).

• **RESULTS:** TEM revealed apoptosis of lens epithelial cells in both two groups. Moreover, we observed lamellar delamination, granular belts in the anterior capsular zone and loss of the subcapsular epithelium cells in the posterior capsular zone, as well as abnormal fibrils located in the central capsular layer only in the sample from the patient with true exfoliation.

• **CONCLUSION:** We suggest that loss of lens epithelial cells and appearance of abnormal fibrils is important in disease developing, and our study supported age-related degeneration is one of causative factors in true exfoliation.

• **KEYWORDS:** true exfoliation; age-related cataract; transmission electron microscopy; apoptosis

**DOI:10.3980/j.issn.2222-3959.2013.04.27**

Shentu XC, Zhu YN, Gao YH, Zhao SJ, Tang YL. Electron microscopic investigation of anterior lens capsule in an individual with true exfoliation. *Int J Ophthalmol* 2013;6(4):553-556

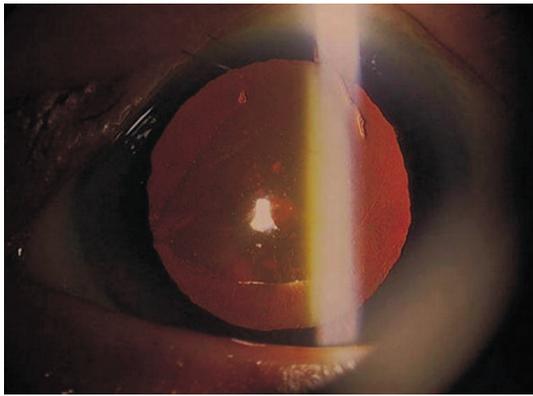
## INTRODUCTION

True exfoliation is a rare and easily overlooked disorder in which the lens capsule is thickened and the superficial portion of the lens capsule splits from the deeper layer and float as a clear thin membrane in the anterior chamber<sup>[1]</sup>. The pathogenesis of this disorder is not clear. Intense infrared radiation, inflammation and trauma are all considered to be the causative factors<sup>[2]</sup>. Besides, age-related degeneration is also suggested to be one cause<sup>[3]</sup>.

We here report a 93 year-old patient with cataract who was confirmed to have a bilateral split arising in the anterior capsule. With the assistance of transmission electron microscopy (TEM), we not only found the capsule lamellar delamination, capsule granular belts and loss of the subcapsular epithelium cells, but also abnormal fibrils in patient's anterior capsule interestingly. The results of TEM provided the strong support for the diagnosis of true exfoliation.

## SUBJECTS AND METHODS

**Subjects** A 93 year-old Chinese man complained of a progressive bilateral painless loss of vision over the preceding 2 years. He did not report any prolonged heat exposure. Instead, he gave a history of working in the post office of Hangzhou, which mainly dealt with paperwork. The patient had apparently never been exposed to toxic substances and had no other ophthalmic history. The best Snellen visual acuity was 20/80 in the right eye and 20/100 in the left. Bilateral nuclear and cortical cataracts were identified, with a scrolled leaf of anterior capsule floating in the anterior chamber in both eyes symmetrically. The peripheral anterior capsule was involved as the result of capsular peeling (Figure 1). The intraocular pressure, optic nerves, and fundi were normal in both eyes. We also enrolled other six age-related cataract patients as controls. The exclusion criteria for controls included: 1) patients who were younger than 85 year old; 2) patients who were suffering from any other ocular diseases, such as corneal disease, glaucoma, uveitis, retinal detachment, optic neuropathy or amblyopia; 3) patients who were suffering with any systemic diseases that might affect treatment, such as diabetes mellitus; 4) patients who were prescribed with any drug that might affect surgery, such as  $\alpha$ -receptor antagonist. These six patients underwent standard phacoemulsification and foldable lens implantation with 5mm continuous curvilinear



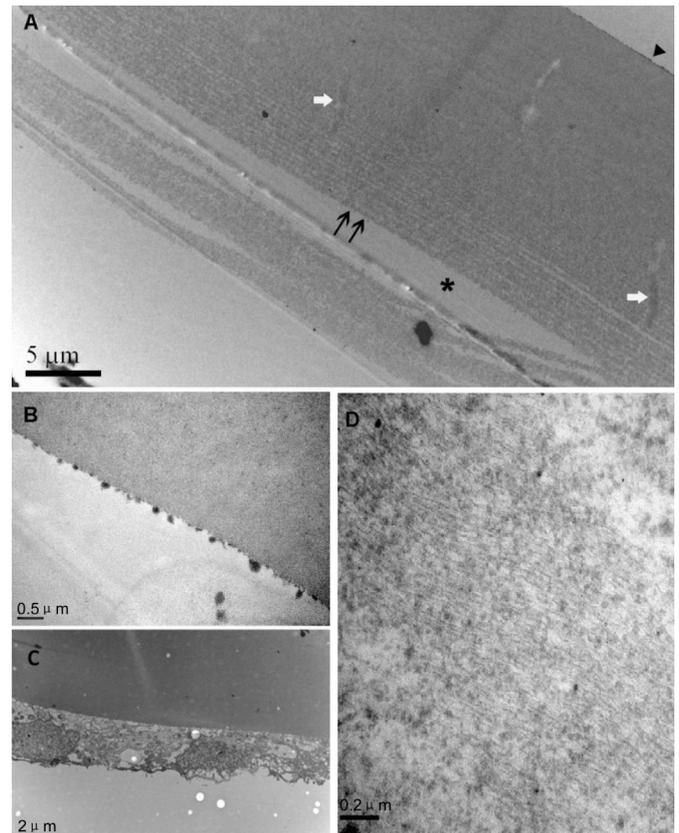
**Figure 1** Retroillumination showing the range of anterior lens capsule peeling.

capsulorhexis(CCC). And we fixed and detected lens anterior capsules under TEM.

**Methods** A routine phacoemulsification was performed. Before CCC, a crescent-shaped split with a capsular flap developed upon the injection of Healon into the left anterior chamber. We used a forceps to do the capsulorhexis, without any trypan blue or indocyanine green visual staining. Most parts of advancing edge of the capsulorhexis bypassed the true exfoliation margin, while a few part of the CCC edge just crossed the true exfoliation margin, for the superficial thin layer separated from the deep layer did little influence to the CCC. Phacoemulsification was performed using the phaco-chop technique and an acrylic intraocular lens was safely inserted in the capsular bag. The patient's vision improved to 20/20 postoperatively. Immediately after excision of the anterior capsular flaps, the capsules were fixed in 2.5% phosphate-buffered glutaraldehyde (pH 7.2) for 60min at 4°C and then in 2% Osmic acid for 90min. The capsule was dehydrated in an ascending methanol series and embedded in Epon 812. Ultra-thin sections of 60nm thickness were stained with uranylacetate and lead citrate for examination under TEM.

## RESULTS

**The Case** TEM revealed that the anterior lens capsule was 21.5µm, of which 13µm was a normal, compared with a remaining 8.5µm outer layer. The anterior capsule zone consisted of 300-400nm thick alternating electron-dense stratified granular belts, which were most evident in the central area of the capsule and disappeared gradually toward the periphery. Certain sections of the posterior area adhered with the lens epithelium cells. The subcapsular lens epithelium cells displayed apoptosis, with cells shrinkage, pyknosis, intracellular vacuoles and gap enlargement between cells. Also, lots of lens epithelium cells were lost in many parts of posterior area, substituted by a homogeneous granular material that was arranged in a linear pattern, which were cell debris. Interestingly, in the capsule, several abnormal fibrils were found to be located in the central capsular layer, and perpendicular to the structural layer (Figure 2).

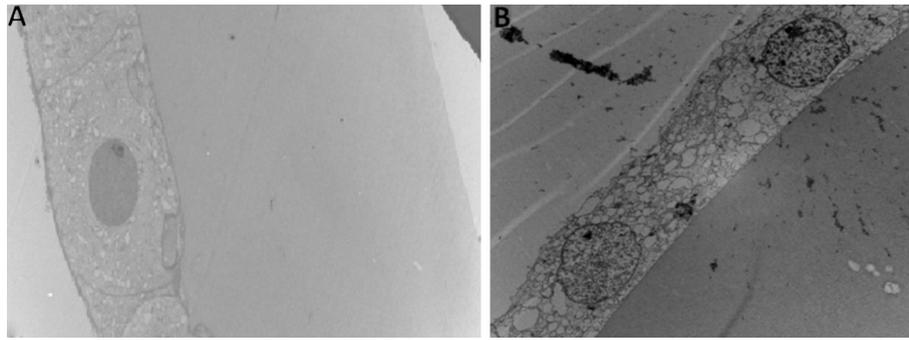


**Figure 2** Abnormal fibrils located in the central capsular layer, and perpendicular to the structural layer A: TEM of the anterior capsule reveals capsule delaminations. The asterisk showed the major cleavage plane along which the capsule split. Double black arrows indicated the granular belts, layered at 300-400nm intervals. The posterior capsular zone is composed of a homogeneous electron-dense material in a linear pattern (black triangle). The white arrows showed abnormal fibrils located in the capsular layer ( $\times 3000$ ); B: Homogeneous electron-dense material on the posterior capsular zone ( $\times 30000$ ); C: Lens epithelium cells undergoing apoptosis with cells shrinkage, pyknosis, intracellular vacuoles and gap enlargement between cells ( $\times 6000$ ); D: Magnified view of abnormal fibrils ( $\times 40000$ ).

**Controls** Mean age of the controls was 87.8 (range, 86-90) year. All of them were revealed to have integrated capsule, with thickness of  $27.2 \pm 1.3$  (range, 26.0-28.6)µm, and no cleavage was found in the capsular layers. One in six controls has signs of apoptosis in lens epithelial cells, which were almost similar with the case, not only cells shrinkage and pyknosis, but also intracellular vacuoles and gap enlargement between cells. Other five controls did not have any sign of apoptosis. There was no epithelial cells' loss in the controls (Figure 3). Characteristics of lens anterior capsules between the true exfoliation case and controls were detailed in Table 1.

## DISCUSSION

True exfoliation (TE) of the crystalline lens is a rare condition in which the lens capsule splits and the anterior layer floats in the anterior chamber. Since Elschnig first described TE of the lens capsule in 1922, there have been at



**Figure 3 TEM of the anterior capsule from patients with age-related cataract** A: Integrated capsule with normal lens epithelial cells ( $\times 2\ 500$ ); B: Lens epithelial cells undergoing apoptosis with cells shrinkage, pyknosis, intracellular vacuoles and gap enlargement between cells as similar as true exfoliation ( $\times 3\ 000$ ).

**Table 1 Characteristics of lens anterior capsules between the true exfoliation case and controls**

Group	Capsule		Lens epithelial cells	
	Cleavage	Abnormal fiber	Apoptosis	Loss
Case	Present	Present	Present	Present
Control	Absent	Absent	1/6 present	Absent

least 40 more TE cases reported. TEM is a good method for exploring the characteristics of true exfoliation. Capsule lamellar delamination, capsule granular belts and lens epithelium cell degeneration are key features for true exfoliation<sup>[4-6]</sup>.

Our TEM study was confirmed previous reports of the partial splitting of the anterior capsule in true exfoliation, cleavages between capsular layers could be observed. In addition, our study observed apoptosis progress with lens epithelial cells shrinkage, pyknosis, intracellular vacuoles and gap enlargement between cells as similar as Ataka's research<sup>[6]</sup>. Interestingly, we also found the same signs in one control. As apoptosis of lens epithelial cells was usually found in age-related cataract, we confirmed that this senile appearance was one common factor between true exfoliation and age-related cataract, but not the specific sign in true exfoliation<sup>[7]</sup>. Meanwhile, this patient had no heat or toxic exposure, nor other ophthalmic history. The obvious feature of him was his age of 93 years old. So we suggested that these senile pathological characteristics may support that aging was one of the causes to true exfoliation.

Furthermore, we obtained a new TEM finding that in some areas of the posterior capsular zone, the lens epithelial cells were totally lost, and instead by some homogeneous granular cell debris which was arranged in a linear pattern. This phenomenon was never found in the controls. The loss of the cells may indicate more severe apoptosis of lens epithelial cells. Apparently, the number of lens epithelial cells in true exfoliation was much less than which in the controls. According to the research before, the lens capsule normally

increases in thickness by the lens epithelial cells secreting and depositing parallel to the capsule surface and at the inner surface, that later push through toward the outer surface<sup>[8]</sup>. Learned from the TEM results, we suggested the fewer amount of lens epithelial cells which resulted from severe age-related degeneration may be one of causes that enough normal lens capsular lamellae failed to form and instead form the abnormally thickened, stratified structure.

Interestingly, we found several abnormal fibrils in the central area of the anterior capsule, perpendicular to the structural layer only in the case of true exfoliation. Fibrils were composed of microfibrils, which were parallel with same intervals, and seemed to be well arranged. Central anterior capsule layers were full of collagens IV<sup>[9]</sup>, these abnormal fibrils destroyed the continuity of original collagen and reduced adherence force there, making collagen there more easily to be fractured. We suggested these fibrils were the collagen degeneration, they may accelerate the splitting layer to fracture, and float in the anterior chamber.

Besides, different from the reports before, we didn't think use of the trypan blue or indocyanine green visual staining of the anterior capsule must be necessary in the capsulorhexis<sup>[10,11]</sup>. Surely trypan blue staining of the capsule permitted the construction of a clearly identifiable CCC, but we considered choosing a normal point outside the extent of the true exfoliation margin as a beginning and carefully pulling the capsule to bypass the abnormal district were the key points to avoid the complication. Furthermore, in our experience, we observed that the delaminated superficial layer was so thin, so if we did the CCC just crossing this abnormal delaminated capsule, this thin superficial layer would be unable to influence the track of capsulorhexis, and the capsular integrity would not be destroyed. Moreover, similar to Radner *et al*<sup>[12]</sup> report before, we also confirmed that careful CCC produced the mildest tissue damage. Under TEM, lens edges obtained by CCC were smooth with no irregularities, and the edges were beveled anteriorly to posteriorly with no

evidence of tearing. We just easily exclude the influence that the CCC made, or the structural destroy during the specimen making.

In our case, all of these TEM findings supported the diagnosis of true exfoliation according to the reports in the literature. Combined with the patient's phenotype, which included a scrolled leaf of anterior capsule floating in the anterior chamber, the diagnosis of true exfoliation was definite. Our study supported aging was one of causative factors in true exfoliation, and we suggested that loss of lens epithelial cells and appearance of abnormal fibrils may be important processes in disease developing.

**REFERENCES**

1 Kulkarni AR, Al-Ibrahim J, Haider S, Elsherbiny S, Scott R. Phacoemulsification in true exfoliation of the lens capsule: a case series. *Eye(Lond)* 2007;21(6):835–837

2 Chen HS, Hsiao CH, Chuang LH, Su WW. Clinicohistopathology of cataract associated with true exfoliation of the lens capsule. *J Cataract Refract Surg* 2011;37(5):969–970

3 Asakage H, Ijichi H, Ishiwata T, Hida T, Fujiwara T, Fukuda M. Report of two cases with idiopathic true exfoliation of the lens capsule—histopathological and electron microscopical study. *Nippon Ganka Gakkai Zasshi* 1994;98(7):664–671

4 Elschmig A. Detachment of the zonular lamellae in glassblowers. *Klin Monatsbl Augenheilkd* 1922;69:732–734

5 Karp CL, Fazio JR, Culbertson WW, Green WR. True exfoliation of the lens capsule. *Arch Ophthalmol* 1999;117(8):1078–1080

6 Ataka S, Kohno T, Kurita K, Wada S, Takahashi Y, Shiraki K. Histopathological study of the anterior lens capsule with a double–ring sign. *Graefes Arch Clin Exp Ophthalmol* 2004;42(3):245–249

7 Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, Spector A, Leib M, Cotliar AM, Weiss M. Lens epithelial cell apoptosis appears to be a common cellular basis for non–congenital cataract development in humans and animals. *J Cell Biol* 1995;130(1):169–181

8 Seland JH. Ultrastructural changes in the normal human lens capsule from birth to old age. *Acta Ophthalmol* 1974;52(5):688–706

9 Kelleys PB, Sadob Y, Duncan MK. Collagen IV in the developing lens capsule. *Matrix Biol* 2002;21(5):415–423

10 Rossiter J, Morris A. Trypan blue vital staining of the anterior lens capsule in the management of cataract in true exfoliation of the lens capsule. *Eye(Lond)* 2005;19(7):809–810

11 Kim KH, Chung ES, Chung TY. Radial extension of capsulorhexis in true exfoliation patient: a potentially hazardous complication. *J Cataract Refract Surg* 2009;35(3):590–592

12 Radner G, Amon M, Stifter E, Nepp J, Diendorfer G, Mallinger R, Radner W. Tissue damage at anterior capsule edges after continuous curvilinear capsulorhexis, high–frequency capsulotomy, and erbium:YAG laser capsulotomy. *J Cataract Refract Surg* 2004;30(1):67–73