Anterior Capsular Plaque in Congenital Cataract: Occurrence, Morphology, Immunofluorescence, and Ultrastructure

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PURPOSE. To study occurrence, morphology, immunofluorescence, and ultrastructural features of congenital anterior capsular plaque (ACP) obtained from pediatric eyes undergoing cataract surgery.

METHODS. Two hundred sixty consecutive pediatric eyes undergoing congenital cataract surgery were enrolled in the present study. Anterior lens epithelium from cataract without ACP and with ACP was collected. Whole mounts of lens epithelium were stained with hematoxylin-eosin. Five-micrometer-thick sections of large ACPs were subjected to immunofluorescence and electron microscopy.

RESULTS. The overall occurrence of ACP in pediatric eyes undergoing congenital cataract surgery was 11.5%. The occurrence of ACP was highest in mature cataract followed by nuclear, lamellar, and mixed cataract. The whole mount of anterior lens epithelium revealed nonplaque and plaque region or ACP. Depending on the area, ACPs can be classified as small, medium, and large. The extracellular matrix of ACP was fibrous and amorphous. It was rich in collagen type I. The cells of the ACP were surrounded by a network of collagen type IV and were positive for α-SMA and α-crystallin. The cells of the ACP were rich in rough endoplasmic reticulum and mitochondria.

CONCLUSIONS. The occurrence of ACP in pediatric eyes undergoing congenital cataract surgery for congenital cataract was 11.5%. ACP was more associated with mature cataract. Epithelial mesenchymal transdifferentiation of lens epithelial cells may be involved in the development of congenital ACP.

The human lens is a highly organized structure consisting of two types of cells: an anterior monolayer of lens epithelial cells (LECs) and a central mass of fiber cells. In the equatorial region of the lens, LECs undergo terminal differentiation to form lens fibers. During this process, cells gradually lose cell organelles and acquire specific proteins like β- and γ-crystallin. This process continues and lack of cell turnover enables the lens to grow throughout life.1–3 Several studies have shown that besides this normal differentiation, LECs also have the ability to undergo epithelial mesenchymal transdifferentiation (EMT) into myofibroblasts and to secrete extracellular matrix (ECM).4–6 EMT in the lens leads to the formation of a distinct opaque region called plaque.7–9 Plaque is a multifocal, dense, white opacity adherent to the internal surface of anterior and posterior lens capsule. Plaques are relatively uncommon in Europe and North America.10,11 They are more often seen in developing countries such as India.7,8,12,13 The mechanism of plaque formation has been described in adults.9,12–15 Studies have not been reported on the occurrence, morphology, and pathogenesis of anterior capsular plaque (ACP) in patients with congenital cataract. Hence, the present study was designed to evaluate the occurrence, morphology, immunofluorescence, and ultrastructural features of congenital ACP.

MATERIALS AND METHODS

Chemicals

Mouse anti-collagen type I, mouse anti-collagen type IV, mouse anti-alpha smooth muscle actin, 4,6-diamino-2-phenylindole (DAPI), phosphate-buffered saline (PBS), and bovine serum albumin were obtained from Sigma-Aldrich (St. Louis, MO). Anti-mouse antibody tagged with AlexaFluor 488 and anti-rabbit antibody tagged with AlexaFluor 546 were obtained from Invitrogen-Molecular Probes (Eugene, OR). Rabbit anti-α-crystallin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). An Epon mounting kit, glutaraldehyde, lead citrate, and uranyl acetate were purchased from Fluka, (Buchs, Switzerland). The remaining chemicals were obtained from Merck (Mumbai, India) and Himedia (Mumbai, India).

Study Population and Sample Collection

The study was performed on 260 eyes of 196 consecutive pediatric patients who were undergoing congenital cataract surgery at Iladevi Cataract and IOL Research Centre between June 2003 and May 2006. The study population comprised children 15 years of age who had congenital cataract. Subluxated cataract and eyes with coexisting intraocular diseases such as uveitis, glaucoma, and persistent hyperplastic primary vitreous were excluded. All eyes underwent preoperative examination with maximum mydriasis. The type of cataract and presence of the ACP was determined with a slit lamp biomicroscope or operating microscope (under anesthesia), depending on the age of the patient and the patient’s cooperation.

Of 260 eyes enrolled for the study, ACP was revealed in 30 eyes of 25 patients and an anterior lens epithelial sample containing ACP was collected from all 30 eyes. Observation of these epithelia under the phase-contrast microscope also revealed the presence of ACP. Of these 30 samples, 10 samples having single ACP with a dimension greater than 1.5 mm were considered as large ACPs and were processed for immunofluorescence and electron microscopy. The remaining 20 samples were processed for morphologic study and image analysis.

The anterior lens epithelium was also collected from 50 eyes with congenital cataract. Four pediatric eyes having clear lens were obtained from local eye bank (Samaria International Red Cross Eye Bank, India). From the 1Iladevi Cataract and IOL Research Centre, Gujarat, India; and the 2Nara Medical College, Nara, Japan. Submitted for publication March 14, 2007; revised April 21 and May 26, 2007; accepted July 10, 2007.

Disclosure: K. Johar, None; A.R. Vasavada, None; K. Tatsumi, None; S. Dholakia, None; B. Nihalani, None; S.S. Lakshmana Rao, None.

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Ahmedabad, India) and were dissected to obtain lens epithelium. These non-ACP samples were collected in PBS (pH 7.4) and processed for morphologic study. The specimens were examined according to the suggestions of the institutional ethics committee and adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained from the subjects.

**Morphology**

Twenty samples with ACP, 50 non-ACP cataract samples, and 4 samples of pediatric clear lenses were processed for the preparation of wholemounts. Each sample was washed in PBS (pH 7.4), spread on a chilled glass slide by using fine forceps to keep the capsule adherent to the slide surface, fixed with Carnoy’s fixative (3:1, methanol-acetic acid), and stained with hematoxylin–eosin and examined under a light microscope (Axioskope 2; Carl Zeiss, Göttingen, Germany). Photographs were taken with a 3CCD camera (Sony, Japan), and images were subjected to image analysis software (Biovis; ExpertVision, Mumbai, India) to evaluate the area of ACP.

**Immunofluorescence**

Five samples containing large ACPs were processed for an immunofluorescence study. Three samples were processed for localization of collagen type I, α-crystallin, and αSMA. These samples were sectioned to obtain 30 cryosections of 5-μm thickness, which were mounted on six silane-coated slides. Among these, two slides each were fixed in methanol for localization of collagen type I and α-crystallin, respectively, whereas the remaining two slides were fixed in 2% paraformaldehyde in PBS for localization of αSMA. The remaining two samples were processed for localization of collagen type IV. They were first fixed in 2% paraformaldehyde in PBS and then processed to obtain 5-μm-thick paraffin-embedded sections. These sections were mounted on silane-coated slides, deparaffinized, hydrated in a graded alcohol series, and washed in PBS. Antigen retrieval from the sections was performed by incubating them in 0.01 M citrate buffer (pH 6.0) at 80°C for 20 minutes in a microwave oven followed by incubation in 1 mg/ml peptide in 0.01 M HCl for 30 minutes at 37°C.18

Both the cryosections and the paraffin-embedded sections were washed thoroughly in PBS. Slides were immersed in PBS containing 1% bovine serum albumin and 1% normal goat serum for 30 minutes. Incubation with primary antibody was performed overnight at 4°C. The primary antibodies used were mouse anti-αSMA (1:300 dilution), rabbit anti-α-crystallin (1:200 dilution), mouse anti-collagen type I (1:1000 dilution), and mouse anti-collagen type IV (1:500 dilution). The sections were then rinsed in PBST (PBS containing 0.05% Tween 20), incubated with the appropriate secondary antibody tagged with AlexaFluor 488 and AlexaFluor 546 (1:200 dilution) containing 0.1 μM DAPI for 1 hour at 37°C, rinsed thoroughly with PBST, and mounted in polyvinyl alcohol-glycerol mounting medium containing 2.5% diazobicycloc-octane (DABCO). The sections were observed by epifluorescence microscope (Axioskope II; Carl Zeiss), and images were taken with a cooled CCD camera (Cohu, San Diego, CA).

**Electron Microscopy**

Five samples containing large ACPs were processed for transmission electron microscopy. The samples were washed in PBS for 10 minutes and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3), then washed in 0.1 M phosphate buffer, and postfixed in 1.2% osmium tetroxide in 0.1 M phosphate buffer (pH 7.3). The samples were dehydrated and were finally embedded in Epon 812 solution. Ultrathin sections of 60 to 70 nm, stained with uranyl acetate–lead citrate, and observed by transmission electron microscope (JEOL, Tokyo, Japan).

**Statistical Analysis**

Statistical analysis of the ACPs by type of cataract was performed using a z-test to determine the proportion of each type. Significance was set at $P < 0.05$. The odds ratio was used to determine the occurrence of ACP in the different types of cataract.

**RESULTS**

**Occurrence**

The mean age of the study population without ACP was 35.3 ± 45.6 weeks and with ACP was 30.7 ± 28.2 weeks. Table 1 shows gender-wise classification of samples by type of cataract and occurrence of ACP. Of 260 eyes enrolled in the present study, 30 samples showed ACP giving an overall occurrence of 11.5%. In the boys the occurrence was 13.0%, and in the girls it was 8%; however, the difference between the boys and girls is not statistically significant. ACP was not encountered in posterior subcapsular, blue dot, and sutural cataracts. Figure 1 represents the occurrence of ACP according to the type of cataract. The occurrence of ACP was highest in mature cataracts (28.84%) and in decreasing order of occurrence in nuclear (13.15%), lamellar (8.69%), and mixed cataracts (5%). Although one of six eyes with cortical cataract had ACP, we did not consider the occurrence of ACP in this type of cataract because of the small sample size. A statistically significant difference was found between the proportion mature cataracts with ACPs and that of both mixed and lamellar cataracts with ACPs ($P < 0.001$). However, this proportion was not statistically significant compared with the proportion of nuclear cataracts with ACPs. A statistically significant difference ($P < 0.001$) was found when the occurrence of ACP in mature cataract (28.84%) was compared with that of all other types of cataract taken together (8.6%), which indicates that the risk of ACP in mature cataract is almost three times higher that that in other types of cataracts. In four cases—three of mature and one of lamellar cataract—ACP was present bilaterally.

**Morphology and Classification**

The ACP appears as a well demarcated multifocal, white opaque area that can be appreciated in contrast with the red glow of the fundus. It is difficult to identify ACPs in white mature cataracts because of the lack of contrast. There may be multiple small ACPs distributed throughout the anterior lens epithelium or a single large ACP that may be located centrally or eccentrically. Microscopic examination of wholemount lens epithelium obtained from clear lenses show a monolayer of cubical LECs adherent to the lens capsule. The LECs were distributed evenly and the nuclei were uniform (Fig. 2A). The LECs in the cataract showed changes such as faintly stained cytoplasm and heterogeneity in the shape and size of the nuclei (Fig. 2B). The nuclei were located unevenly and scattered, superimposed cells were found (Fig. 2C). The anterior lens

<table>
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<tr>
<th>Cataract</th>
<th>Total Male</th>
<th>Female</th>
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<tr>
<td>Nuclear</td>
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<td>Cortical</td>
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<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Lamellar</td>
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<td>50</td>
<td>19</td>
<td>6</td>
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<tr>
<td>Mixed</td>
<td>62</td>
<td>43</td>
<td>19</td>
<td>3</td>
</tr>
<tr>
<td>Mature</td>
<td>52</td>
<td>39</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>PSC</td>
<td>12</td>
<td>7</td>
<td>5</td>
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<td>Sutural</td>
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<td>4</td>
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<td>0</td>
</tr>
<tr>
<td>Blue dot</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Membranous</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>185</td>
<td>75</td>
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**TABLE 1. Classification of Samples by Type of Cataract and Occurrence of ACP**
epithelium obtained from eyes having ACP had two distinct regions: nonplaque and plaque (ACP) region. The nonplaque region had LECs similar to the LECs of samples obtained from non-ACP cataract samples. Alterations such as faintly stained cytoplasm, heterogeneity in the shape and size of nuclei, and superimposed cells were found (result not shown). Occasional mitotic figures were noted in the cells located at the periphery of the ACP (Fig. 2D).

Based on the area, ACPs were classified as small (<0.1 mm²), medium (0.1–0.3 mm²), and large (>0.3 mm²). Depending on the area of ACP present in the sample, the samples were also classified based on associated cataract type (Table 2). Five of 30 samples had multiple ACPs, and these samples were allocated to the classes based on the area of the largest ACP present in that sample. Ten samples having ACPs of a minimum length of 1.5 mm as measured by a phase-contrast microscope and later processed for immunofluorescence and electron microscopy were also included in the large category of ACP. Four samples had multiple small ACPs (Fig. 3A) and were often associated with nuclear and lamellar cataracts. Two samples had medium ACPs along with few small ACPs, whereas the remaining nine samples had a single medium ACP (Fig. 3C).

Large ACPs were always single (Fig. 3E). Both medium and large ACPs were associated with nuclear and lamellar cataracts. Two samples had multiple small ACPs (Fig. 3A) and were often associated with nuclear and lamellar cataracts. Two samples had one medium ACP along with few small ACPs, whereas the remaining nine samples had a single medium ACP (Fig. 3C). Large ACPs were always single (Fig. 3E). Both medium and large ACPs were usually associated with mature and mixed cataracts. The smaller ACPs consisted of a large number of cells with round nuclei and scanty ECM (Fig. 3B). As the area of ACP increased, the quantity of ECM also increased, with consequent decrease in number of cells (Figs. 3D, 3F).

**Immunofluorescence and Ultrastructure Studies**

Both the immunofluorescence and ultrastructural studies were conducted to characterize cells and ECM in eyes with ACP. The ECM was abundant in collagen type I (Fig. 4). Ultrastructurally, the ECM appeared both fibrous and amorphous. The fibers in the ECM were broad, ribbonlike, and arranged in bundles. The fibers showed periodicity of bands at 60 to 70 nm, which is a characteristic of collagen type I (Fig. 5A). Amorphous ECM consisted of debris containing electron-dense, small, irregular particles; randomly arranged filaments; and electron-dense vesicles (Fig. 5B). The cells of ACPs were surrounded by a multilamellar basement membrane (Fig. 5C). An immunofluorescence study also suggests that cells of the ACPs were surrounded by a network of collagen type IV (Fig. 4). Under transmission electron microscopy the cells of the ACPs showed abundant rough endoplasmic reticulum (RER) and large, round, amorphous mitochondria (Fig. 5D). Immunofluorescence study suggested that the cells of ACPs are positive for αSMA which is a marker of myofibroblasts (Fig. 4). The cells were also positive for αA-crystallin, a protein found in the LECs (Fig. 4). These characteristics were found throughout the ACPs.

**Discussion**

Childhood blindness in the developing countries, particularly in the Indian subcontinent, is high, and cataract is one of the major causes. It has far reaching effects on educational, professional, personal, and social aspects of the child and his family. Our study shows a 11.5% occurrence of ACP in cases of congenital cataract. The ACP occurrence was highest in the mature cataract, perhaps due to the chronic biochemical changes in mature cataract that trigger LECs to form an ACP. The mature cataract epitomizes the most severe form of cataract, the high occurrence of ACP may suggest that it is related to the severity of the cataract. The presence of ACP in some cases of cataract other than mature cataract is not known.

EMT is a dynamic process in which cells change from the epithelial state of differentiation into a mesenchymal phenotype. EMT is a well-recognized mechanism for dispersing cells in vertebrate embryos, forming fibroblasts in injured tissues, and initiating metastasis in tumors. The ACP consisted of cells and ECM. The presence of collagen type I in the ECM and αSMA in the cells suggests the involvement of EMT of LECs in the development of ACP. Previous studies have demonstrated that the ACPs in adult anterior subcapsular cataract and anterior polar cataract also possess myofibroblasts and a...
Our observations showed the presence of A-crystallin, which is a major protein of the lens cells. The cells of the ACPs were also surrounded by collagen type IV, suggesting active synthesis of this protein by the cells. Unlike the lens cells, cells in the ACPs were rich in ribosome and RER, commensurate with the increased...
activity needed for transdifferentiation and for secretion of ECM components. These findings imply that cells of ACPs are derived from LECs that have undergone EMT and have formed active myofibroblasts while retaining some of their LEC characteristics.

Earlier work on adult ACPs suggested two phases in their development. In the first phase, cells undergo rapid proliferation, and in the second phase they transdifferentiate, secret ECM, and finally degenerate. We have found morphologic alterations such as faintly stained cytoplasm and heterogeneity in the shape and size of nuclei in the LECs of anterior lens epithelium obtained from eyes with congenital cataracts. Scattered superimposed LECs were found in the lens epithelium of cataract samples with ACP and in those without. Earlier, we also reported superimposition of LECs in both experimental and adult human cataract. We believe that the alteration in the LECs may trigger proliferation of LECs in certain regions, which leads to superimposition of cells. Later on, these superimposed cells may undergo rapid division and EMT to form the smaller ACP. The presence of mitotic figures at the periphery accounts for the peripheral growth of ACP, and this growth may explain the origin of larger ACPs, due to growth or fusion of smaller ACPs. It was interesting to note that the area of ACP is proportional to the amount of ECM.

The expression of αSMA and secretion of ECM containing collagen type I were observed in adult human plaques and animal plaque. Similar observations were also noted in the anterior and posterior capsular opacification (PCO) after cataract surgery as a wound-healing response of LECs. However, congenital ACP takes place without any trauma. The higher occurrence of ACP in pediatric patients may be due to the high proliferation capacity of pediatric LECs. The pediatric patients also show high occurrence of PCO, suggestive of increased capacity of pediatric LECs for cell division and transdifferentiation.

In conclusion, the present study showed an alarming occurrence of 11.5% ACP in eyes with congenital cataract. The occurrence was highest in white mature cataract. The presence of αSMA in the cells and ECM containing collagen type I may indicate the involvement of EMT in the formation of congenital ACP. The transdifferentiated cells of ACPs retained LEC properties such as α-crystallin and were surrounded by collagen type IV. An attempt also was made to classify ACPs based on their morphologic characteristics.

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