Original Article

Anterior segment alterations in congenital primary aphakia—a clinicopathologic report of five cases

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Purpose: To report the clinicopathological features of corneal buttons in patients with congenital primary aphakia. **Methods:** Five corneal specimens of five patients with congenital primary aphakia who underwent penetrating keratoplasty (PKP) were studied by light microscopy, and immunohistochemistry with anti-smooth muscle (SMA) antibody. **Results:** All patients were born from consanguineous parents. Of the five, two patients were identical twins. All eyes were microphthalmic. In four patients, congenital primary aphakia was bilateral and in one patient (Patient 3), it was unilateral. PKP failed in all eyes due to hypotony. Histologically, Bowman's layer was absent in all specimens. The corneal stroma was thin; however, the stromal collagen showed thick and irregularly arranged fibers with neovascularization in all eyes. Descemet's membrane and the corneal endothelium were absent in all specimens. In three specimens, atrophic iris tissue without dilator muscle was adherent to the posterior corneal surface. In addition, anteriorly displaced hypoplastic ciliary body and/or pigmented and non-pigmented ciliary epithelium were attached to the posterior corneal surface in three of the five specimens. SMA staining demonstrated disorganized ciliary muscle in one case. SMA-positive stromal keratocytes demonstrated their myofibroblast nature. **Conclusion:** The corneal findings in congenital primary aphakia are similar to that seen in other causes of congenital corneal opacification. The anteriorly displaced hypoplastic ciliary body that was partially excised during keratoplasty explains the ocular hypotony in these eyes.

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Congenital primary aphakia (*OMIM* 610256) can be isolated or present as part of a complex anterior segment abnormality that includes microphthalmia, absence of the iris, anterior segment aplasia, and/or sclerocornea, and/or it may be associated with multiple somatic abnormalities. The condition is thought to result from an event during the 4th or 5th week of fetal development, which prevents the formation of lens structures in the eye.[1] The failure of lens development results in dysgenesis of the ocular anterior segment. The posterior segment changes include hypoplastic ciliary processes and an abnormal vitreous.[2] Majority of the eyes have microphthalmos. As a result of severe anterior segment dysplasia, many eyes develop glaucoma and an anterior staphyloma at a later age. Because of hypoplastic ciliary process and an abnormal vitreous, keratoplasty is unsuccessful in these eyes and hence avoided.[3,4]

The purpose of this report was to examine the histological changes in the cornea of patients with congenital primary aphakia who underwent penetrating keratoplasty (PKP), and to correlate these findings with the clinical findings, to explain some of the clinical features and reasons behind poor outcomes of PKP.

Methods

The study included five patients who were clinically and histologically diagnosed with congenital primary aphakia. Of the 83 patients diagnosed with corneal opacity with congenital primary aphakia between 2008 and 2018, five eyes underwent PKP. The clinical diagnosis of congenital primary aphakia was made based on the typical silvery white/bluish haze of the cornea as shown in the representative images [Fig. 1]. B Scan ultrasonography performed by an experienced ultrasonographer confirmed the complete absence of the crystalline lens. The absence of lens was also noticed intraoperatively and was documented in the surgical notes. The keratoplasty in the five patients was performed by two experienced corneal surgeons during the years 2010–12, when the management guidelines of this condition were not well defined. Full thickness corneal buttons obtained during PKP were submitted for histopathologic analysis.

The corneal buttons were bisected carefully and processed. Five microns thick sections of formalin fixed, paraffin embedded tissues were stained with hematoxylin and eosin (H and E) and periodic acid schiff (PAS) stains. Immunohistochemistry (IHC) was performed on corneal buttons of patient 1, 2, and 4

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for smooth muscle actin (Dako Inc, Santa Clara, CA, USA, ready to use, monoclonal mouse anti-human smooth muscle antigen, clone 1A4, 1-h incubation time) using indirect immunoperoxidase technique with DAB as chromogen. IHC was done to demonstrate the smooth muscle in the ciliary body seen in some specimens and the myofibroblastic nature of corneal keratocytes.[5] The thickness of the corneal stroma was measured using a caliper tool software (of Jenoptik ProgRes C3 3.2 MP CCD Digital Microscope Camera mounted on Olympus BX51 microscope) in two specimens (of Patient 1 and 2) where the stroma was not disorganized. The stromal thickness was assessed in three different areas and the average of the three measurements was noted.

Results

Table 1 summarizes the clinical and histopathological features of the specimens studied. Fig. 1a–e shows the clinical photographs of the patients 1, 2, 3, and 5 (pictures of patient 4 not available). Fig. 2 shows the spectrum of histopathological findings of the corneal specimens.

In all eyes, the limbus was ill defined, and cornea was small (<7-8 mm approximately in horizontal axis) with a bluish discoloration. B Scan revealed echo free vitreous and normal disc excavation in five eyes; and choroidal coloboma in one eye (Patient 4). The intraocular pressures (IOPs) were recorded in normal range (9–12 mm Hg) under anesthesia.

Of the five, four patients had a history of parental consanguinity. Patient 1 and 2 were identical twins born to consanguineous parents. Dysmorphic features were absent in all patients. All five eyes had microphthalmos. In four patients, congenital primary aphakia was bilateral and in one patient (Patient 3), it was unilateral. In patients 1 and 2 (identical twins), the other eye had an anterior staphyloma. In patient 3, the contralateral eye had a localized corneal opacity with iridolenticular adhesions, consistent with features of Peters anomaly. In patients 4 and 5, the contralateral eye had features that were identical to the operated eye.

The mean age at PKP was 6 months. The left eye was operated upon in three patients and the right eye in two patients. The placement of the trephine was guided by the ill-defined limbus and the trephine was placed after center of the cornea was identified using the horizontal and vertical axis as a guide. The mean size of recipient bed trephination was 5.25 mm (range 5–6) mm. The donor graft was oversized by 1 mm and sutured with 12–16 interrupted 10-0 Nylon sutures. Although the graft was clear for initial 1–2 months, hypotony and graft failure ensued eventually in all five eyes. Fundus examination performed in the two identical twins at 1-month postoperatively after the graft was clear, revealed clear vitreous, normal disc and attached retina.

The histological features seen in the five corneal buttons are summarized in Table 1. Fig. 2 shows the representative histological findings. In brief, in some specimens the corneal epithelium was largely normal, but some demonstrated mild variable surface keratinization, and focal areas of basal cell edema. Bowman's layer was absent in all specimens [Fig. 2a, upper left].

The corneal stroma appeared thin (290 and 300 microns in two specimens) with stromal collagen bundles that were thick and irregularly arranged in all eyes [Fig. 2a and b; upper panel]. The corneal keratocytes showed plump nuclei [Fig. 2a and b; upper panel] and showed positive labeling with smooth muscle actin antibody [Fig. 2d; lower right]. Descemet membrane and the corneal endothelium were absent in all specimens in multiple sections that were examined [Fig. 2a and b; upper panel].

In three specimens (Patient 2,3 and 4), iris tissue was adherent to the posterior corneal surface [Fig. 2b, upper right]. The dilator muscle was absent on H and E stained sections [Fig. 2b; upper right] and by smooth muscle actin immunohistochemistry (not shown).

In addition, ciliary body and/or pigmented and non-pigmented ciliary epithelium were identified in three of the five specimens (Patient 1, 2, and 4) [Fig. 2a; upper left and Fig. 3]. In patient 1, the ciliary body showed extreme anterior displacement along with the iris. An area of the ciliary body which showed collagen fibers adherent to the sclera was identified as the rudimentary scleral spur. In this region, the trabecular meshwork beams and Schlemm's canal was not identified at the anterior edge of the ciliary body/scleral spur where these structures should have been normally located [Fig. 2a; upper left]. In the specimen (Patient 1) that demonstrated rudimentary ciliary body, disorganized remnants of smooth muscle actin positive ciliary muscle were noted [Fig. 2d; lower right]. In other buttons, hypoplastic ciliary body with dilated vessels and atrophic stroma lined the posterior corneal surface devoid of Descemet membrane [Fig. 2a; upper left].

An intraocular epithelial implantation cyst enveloped by atrophic iris pigment epithelium was seen in the anterior segment in one patient [Fig. 4]. The epithelium did not demonstrate features of lens epithelium.

Discussion

This study describes the clinical features and histopathological features in five patients who underwent PKP for congenital aphakia. Few studies have described the histopathological features in this condition.[6,7] Our study adds additional clinical and histological features to this rare condition and also attempts to explain the pathogenesis of the accompanying anterior segment dysgenesis in this condition.

The bluish/silvery white clinical appearance of the cornea is a characteristic feature seen in eyes with congenital primary aphakia and is well recognized amongst pediatric cornea ophthalmologists, though not well documented in the literature. Based on the histological features of the corneal buttons, we suggest that this appearance is likely an optical phenomenon resulting from the thin cornea stroma and disorganized stromal collagen lamellae. The disorganized stromal lamella seen in these corneas are similar to that described in other congenital corneal stromal disorders.[7] However, in contrast to other congenital corneal opacities such as isolated sclerocornea where the corneal stroma can be normal or thicker, 2/5 corneas on histology were thinner as was appreciated intraoperatively, and confirmed on histological measurement. We did not perform ultrasonic pachymetry in these five patients; however, in most of the other patients with congenital primary aphakia seen later at our center, we have documented a thinner cornea stroma by ultrasonic pachymetry (unpublished data). The ill-defined limbus and small corneas in congenital aphakia make it difficult to clinically define the relevant anatomical landmarks during PKP. The average trephination in our cases was 6 mm. Despite the small corneal button, histology of corneal specimens revealed that the iris stroma (in 2 specimens) and ciliary body (in

Table 1: Congenital primary aphakia-clinical and histological features

Patient No	Age at PKP/ Gender	Eye	Clinical features	IOP (mm Hg)	B scan* (axial length)	Recipient bed trephination (mm)	Epithelium	Bowman's layer	Stroma	DM-endothelium	Iris	Ciliary body/ epithelium
1	6 mo/ male	Right	Corneal opacity (Bluish hue), ill-defined limbus	10	13 mm	5	Mild variable keratinization	Absent	Thickened collagen bundles, vascularization, thickness 290 microns	Absent	Not present in specimen	Hypoplastic CB with disorganized ciliary muscle Pigmented and non-pigmented ciliary epithelium
2	6 mo/ male	Right	Corneal opacity (Bluish hue), ill-defined limbus	12	13 mm	5	Mild variable keratinization, epithelial hyperplasia	Absent	Thickened collagen bundles, vascularization, thickness 300 microns	Absent	Iris stroma atrophic pigment epithelium present, absent dilator muscle	Hypoplastic CB, absent ciliary muscle. Pigmented and non-pigmented ciliary epithelium.
3	5 mo/ female	Right	Corneal opacity (Bluish hue), ill-defined limbus	9	13 mm	5.25	Mild variable keratinization	Absent	Thickened collagen bundles, vascularization	Absent	Iris stroma atrophic pigment epithelium present, absent dilator muscle	No CB noted in the specimen
4	4 mo/ male	Right	Corneal opacity (Bluish hue), ill-defined limbus	10	13 mm choroidal coloboma	6	Mild variable keratinization, focal basal cell hydropic degeneration	Absent	Thickened collagen bundles, vascularization	Absent	Atrophic Iris with epithelial implantation cyst, absent dilator muscle	Hypoplastic CB with disorganized ciliary muscle Pigmented and non-pigmented ciliary epithelium
5	6 mo/ female	Left	Corneal opacity (Bluish hue), ill-defined limbus	12	13 mm	5	Mild variable keratinization	Absent	Thickened collagen bundles	Absent	Not present in specimen	No CB noted in the specimen

Mo: Months, IOP: Intraocular pressure, CB: Ciliary body, DM: Descemet membrane, *measured manually on B scan images

3 specimens) were adherent to its posterior corneal surface. The anterior location of the ciliary body and poorly developed iris is a common feature in many anterior segment dysgenesis and congenital glaucoma.[8] In a normally developing eye, the outer corneoscleral collagen grows faster than the uveal tract during the 3rd trimester. This results in posterior migration of the ciliary body to a location behind the scleral spur in the postnatal period.[9] We suggest that a similar mechanism in congenital primary aphakia results in arrest of posterior migration of the ciliary body leading to the anterior located ciliary body and iris. The histological features of the ciliary body noted in our cases are consistent with earlier reports of ciliary body hypoplasia in congenital primary aphakia.[2,6,7] To further add to this finding, we noted the preservation of both layers of the ciliary epithelium and layers of the pigmented iris epithelium. In addition, we noted absent or disorganized ciliary muscle, absent or poorly developed iris stroma and

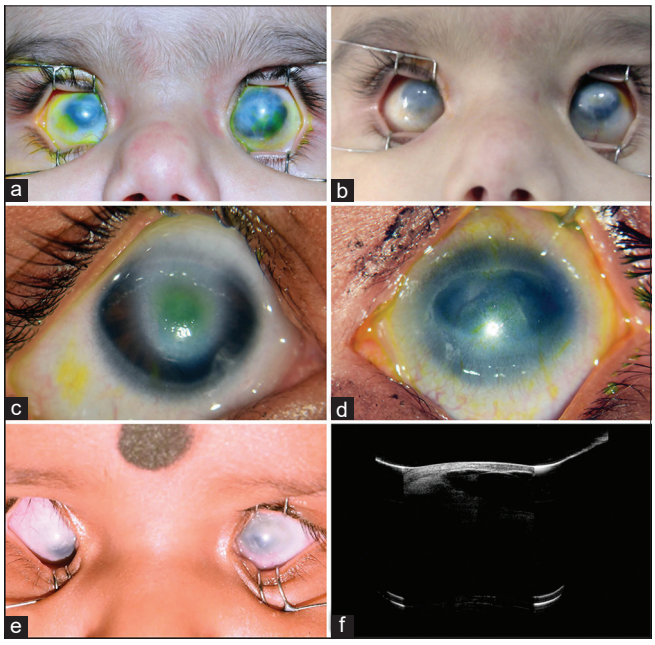

Figure 1: Clinical features of the patients. (a and b) Digital photographs of the identical twins (Patient 1 and 2). The right eye of both shows the silvery white appearance of the cornea; while the left eye of both siblings had complete anterior staphyloma. (c) Digital photograph of the right eye of a Patient 3 who had localized corneal opacity with iridocorneal adhesions, with presence of lens echo (B scan ultrasonography). The clinical picture is corroborative with the traditional diagnosis of Peters anomaly. (d) Digital photograph of the left eye of the same patient showing an ill-defined limbus, bluish hue of the cornea suggestive of congenital primary aphakia in this eye. B scan ultrasonography confirmed the absence of lens in this eye. (e) Digital photograph of the Patient 5 showing silvery white appearance in both corneas. (f) Ultrasound Biomicroscopy (UBM) of the left eye of the Patient 5 showing absence of lens echo (right eye had a similar feature on UBM)

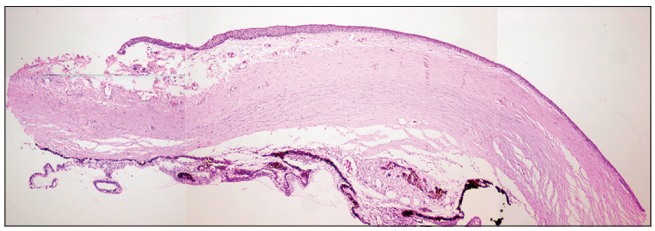

Figure 3: Photomicrograph at low magnification showing the thin, disorganized corneal stroma with atrophic ciliary body and epithelium attached to the posterior surface. Note the sharp edges of corneal trephination (Montage; hematoxylin and eosin; original magnification 4×)

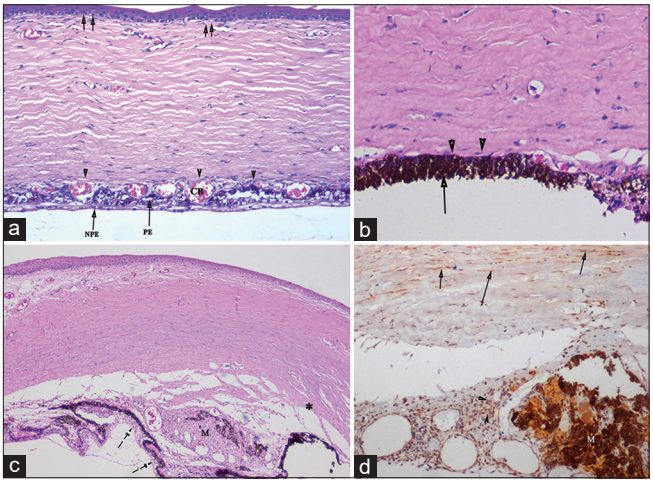

Figure 2: Histopathological features of corneal buttons in Congenital Primary Aphakia. (a): Patient 1. Photomicrograph showing the cornea in congenital primary aphakia: the epithelial maturation appears normal with absence of Bowman's layer (double arrows). The corneal stromal collagen is thick and irregularly arranged with stromal neovascularization. Descemet membrane is absent (arrows). Atrophic ciliary body (CB) with vessels lined by pigmented (arrow PE) and non-pigmented ciliary epithelium (arrow NPE) in noted adherent to the posterior corneal surface. (Hematoxylin and eosin; original magnification 10×). (b): Patient 2. Photomicrographs showing disorganized corneal stroma with two layers of pigmented epithelium consistent with iris epithelium (arrow posterior corneal surface) adherent to the posterior corneal surface devoid of corneal endothelium and Descemet membrane (arrowhead) (hematoxylin and eosin; original magnification 4×). (c): Patient 1. Photomicrograph at low magnification showing central cornea, with ciliary body attached anteriorly to the cornea with a rudimentary scleral spur (arrow). Note the absence of trabecular meshwork and Schlemm's canal in the region. The ciliary body is poorly developed with disorganized muscle (M). However, the pigmented and non-pigmented layers of the ciliary epithelium is well-developed (hematoxylin and eosin; original magnification 4×). (d): Photomicrograph showing alpha smooth muscle actin staining. Note positive staining in the rudimentary ciliary muscle (M). Also note smooth muscle actin-positive myofibroblasts in the corneal stroma (arrows) and spindle cells in the ciliary body stroma (arrowheads). (Diaminobenzidine chromagen; original magnification 4×)

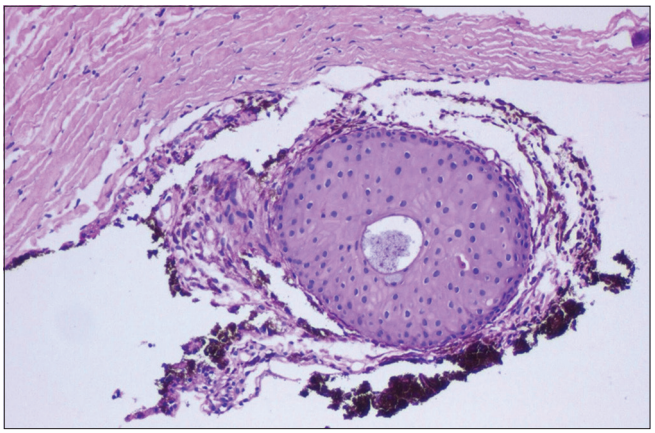

Figure 4: Patient 4. Photomicrograph showing epithelial implantation cyst in the anterior chamber enveloped by atrophic iris tissue. (Hematoxylin and eosin; original magnification 10×)

absent dilator muscle accompanied by an absence of Descemet membrane, corneal endothelium and in one case absence of trabecular meshwork and Schlemm's canal was noted where the ciliary body show marked anterior displacement. The preservation of the anterior ocular neuroepithelium (iris pigment epithelium and ciliary epithelium) suggests that this dysgenetic process does not affect the neuroepithelium of the optic vesicle.[10] However, the variable absence of iris stroma, Descemet's membrane, and corneal endothelium, trabecular meshwork and abnormal corneal stroma suggests that mesodermal and neural crest cell migration and development in the anterior segment is significantly altered in this disorder. It is likely that these processes were altered at a stage when there was a failure of the surface ectoderm to form the lens placode/vesicle resulting in aphakia and severe disruption of development of neural crest and mesodermal structures that form the cornea (keratocytes/collagen, corneal endothelium/Descemet's membrane), iris (stroma), trabecular meshwork, and ciliary body (muscle).[1] All the eyes were microphthalmic as was reported in congenital primary aphakia and other complex anterior segment dysgenesis.[2,6,11]

The thick disorganized corneal stromal fibers with stromal vascularization is similar to what is seen in other conditions with congenital corneal opacification.[7] The smooth muscle actin positive keratocytes indicates the myofibroblastic nature of these cells that is also noted in corneal scarring and corneal keloids.[5]

The histopathological findings point to some factors that might play a role in the poor outcomes after PKP in this condition. The two major anterior segment associations in these patients are glaucoma preoperatively and hypotony following PKP. The absence of trabecular meshwork and Schlemm's canal where ciliary body was identified is similar to what is seen in Peters anomaly and other anterior segment dysgenesis.[7,8] The presence of intact secretory ciliary epithelium lining a hypoplastic ciliary body suggests there is sufficient aqueous humor secretion by these ciliary processes, and might explain the elevated IOP we have observed in the course of follow-up in some of our other patients with congenital primary aphakia. In the patients in this series, the IOP was within normal range, suggesting that there might have been decreased aqueous humor secretion from the ciliary epithelium/hypoplastic ciliary body in the presence of an aberrant outflow pathway. During PKP removal of the anteriorly inserted ciliary body along with ciliary epithelium likely caused further exacerbation of the hyposecretion leading to ocular hypotony. Post-keratoplasty fundus examination at 1 month in the two identical siblings showed a normal disc and attached retina, suggesting that retinal detachment was not the cause of hypotony. Based on these observations, one could speculate that if careful anterior segment ultrasound sonography delineates the ciliary body in its normal location, such patients would be less likely to develop hypotony if a PKP is planned. This hypothesis needs to be tested clinically.

Congenital primary aphakia has been described with systemic syndromes such as Waardenburg's syndrome and trisomy 18. The five patients described here did not have systemic findings associated with these syndromes. Valleix *et al*.[12] reported a consanguineous family in which three siblings had bilateral aphakia, microphthalmia, and complete agenesis of anterior segment and on genetic study showed homozygous mutations in *FOXE3*. It is believed that a mutation in the domain of PAX6 expressing surface ectoderm that forms the lens placode could be the defining event leading to congenital primary aphakia.[13,14] We did not perform genetic study in our patients. But in 4/5 patients reported here, there was a history of parental consanguinity.

Conclusion

To conclude, based on the clinical and histological findings, the management of the corneal opacification in these patients should be conservative. Careful imaging of the anterior segment should be used to determine intervention. Periodic follow-up for assessment of IOPs and treatment with anti-glaucoma medications is warranted to safeguard the limited vision in these eyes.

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Conflicts of interest

There are no conflicts of interest.

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