Unilateral or Asymmetric Pseudoexfoliation Syndrome?

An Ultrastructural Study

Thomas Hammer, MD; Ursula Schlötzer-Schrehardt, MD; Gottfried O. H. Naumann, MD

Background: Clinically, most patients with pseudoexfoliation (PEX) syndrome reveal only unilateral ocular involvement. However, the generalized nature of the disorder suggests that PEX syndrome is clinically asymmetric rather than strictly unilateral.

Objective: To perform an ultrastructural study of the contralateral eyes in patients with unilateral PEX syndrome.

Methods: Five pairs of donor eyes with slitlamp microscopic, macroscopic, and light microscopic evidence of unilateral PEX syndrome and 6 normal control eyes were investigated by transmission electron microscopy and light and electron microscopic immunohistochemistry using antibodies against the human natural killer (HNK-1) epitope and against latent transforming growth factor β1–binding protein, both markers for the identification of PEX deposits.

Results: Ultrastructural alterations were observed in anterior segment tissues of all apparently not involved fellow eyes. These included (1) deposits of typical PEX fibrils on the iris and ciliary epithelia and in the dilator muscle of the iris; (2) increased accumulation of extracellular matrix, including microfibrils and reduplicated basement membrane material in the periphery of iris vessels, in the dilator muscle and in the juxtacanalicular tissue of the trabecular meshwork; and (3) degenerative changes of the iris pigment epithelium and dilator muscle cells. Latent transforming growth factor β1–binding protein– and HNK-1–positive deposits indicating PEX material accumulations were detected in the periphery of iris vessels and in the dilator muscle in all affected and contralateral eyes, but not in the control eyes.

Conclusions: These subclinical alterations of contralateral eyes in clinically so-called unilateral PEX syndrome support the concept that PEX syndrome is a generalized basically bilateral disorder with a clinically marked asymmetric manifestation. The iris changes may account for the clinical signs characteristic of early stages, such as melanin dispersion, peripupillary atrophy, trabecular meshwork pigmentation, and insufficient asymmetric mydriasis. The findings should be considered in the clinical management of the patients.

Clinical Relevance: In view of the fact that PEX syndrome is the most common identifiable cause of open-angle glaucoma worldwide and as it is an important risk factor for a wide spectrum of ocular complications, particularly during cataract surgery, the potential involvement of both eyes in the PEX process is of clinical significance.


The pseudoexfoliation (PEX) syndrome is a degenerative fibrillopathy characterized by the production and accumulation of an abnormal extracellular fibrillar material in the anterior segment of the eye and in various extraocular tissues. Clinically, ocular involvement is described as unilateral in 48% to 76% (ie, most) patients.1-3 In these apparently monocular cases, the fellow eye often has abnormal aqueous humor dynamics,4 a higher intraocular pressure,5 and more pronounced trabecular meshwork pigmentation6 compared with normal eyes. Clinically, unilateral involvement is often a precursor to bilateral involvement, and progression to bilateral involvement was reported in up to 50% of patients within 5 to 10 years after diagnosis.6,7 Patients with bilateral involvement tend to be older and tend to have a higher prevalence of glaucoma than those with a unilateral manifestation.8-10

Recent studies11-13 have demonstrated that PEX syndrome is a systemic process with a wide distribution of PEX material deposits in the body, including the skin, meninges, lungs, heart, and other visceral organs. In accordance, typical PEX accumulations have been detected by electron microscopy in the conjunctiva and in
MATERIALS AND METHODS

TISSUE

Anterior segment tissues were obtained from 5 pairs of donor eyes (mean ± SD age, 74.3 ± 10.0 years; 4 women and 1 man) that revealed PEX deposits on anterior segment structures in only 1 eye by slitlamp microscopic, macroscopic, and light microscopic investigations. The diagnosis of unilateral PEX syndrome was made postmortem by slitlamp microscopic observation of PEX deposits on the anterior lens surface and was subsequently confirmed by macroscopic and light microscopic observation of PEX material on the posterior iris surface, the ciliary processes, the lens, and the zonules. The slitlamp microscopic diagnosis of PEX syndrome had prevented the excision and transplantation of the corneas because of the known PEX-associated corneal endotheliopathy.

Six normal-appearing donor eyes (mean ± SD age, 75.0 ± 7.3 years) without any known ocular disease were used as controls.

ELECTRON MICROSCOPY

The tissue specimens were fixed 2 1/2 to 19 hours postmortem in a solution of 4% paraformaldehyde and 1% glutaraldehyde in a 0.1M phosphate buffer (pH, 7.4), and were embedded in epoxy resin (Epon 812) using a standard protocol. The semithin sections were stained with toluidine blue and the ultrathin sections with uranyl acetate and lead citrate, and examined with a transmission electron microscope (model 906E; LEO, Oberkochen, Germany).

IMMUNOHISTOCHEMISTRY

Because previous studies have shown that antibodies against the carbohydrate epitope HNK-1 and against LTBP-1 are suitable markers for identifying PEX deposits in ocular tissues, we investigated the immunolocalization of HNK-1 and LTBP-1 on the light and electron microscopic level.

For indirect immunofluorescence, tissue specimens were embedded in orithine carbamyltransferase compound and snap-frozen in a combination of isopentane and liquid nitrogen. Cryostat sections (5-7 μm) were fixed in cold acetone, incubated in a 10% solution of normal goat serum in phosphate-buffered saline, and then incubated in primary antibody (polyclonal LTBP-1; Pharmingen, Hamburg, Germany) diluted 1:750 in phosphate-buffered saline overnight at 4°C. Antibody binding was detected by incubating the sections with an Alexa-488–conjugated secondary antibody (Molecular Probes, Leiden, the Netherlands) diluted 1:100 in phosphate-buffered saline for 30 minutes at room temperature.

For postembedding immunogold labeling, tissue specimens were fixed in a freshly made solution of 4% paraformaldehyde and 0.1% glutaraldehyde in a 0.1M cacodylate buffer (pH, 7.4) for 1 to 2 hours at 4°C. After being rinsed, the specimens were dehydrated serially to 70% ethanol at −20°C and embedded in LR White (London Resin Co Ltd, Woking, England) resin using a standard protocol. After blocking unspecific binding sites with 0.5% ovalbumin and 0.5% fish gelatin, the ultrathin sections were incubated in primary antibody solution (monoclonal HNK-1; Becton Dickinson, Erembodegem-Aalst, Belgium) diluted 1:50 in Tris-buffered saline with 0.5% ovalbumin overnight at 4°C. Antibody detection was performed with a 10-nm gold-conjugated secondary antibody (BioCell International, Cardiff, Wales) diluted 1:30 in a combination of Tris-buffered saline, ovalbumin, and gelatin for 1 hour at room temperature. After being rinsed, the sections were stained with uranyl acetate and examined with an electron microscope (model 906E; LEO).

RESULTS

Under a dissecting microscope, all involved eyes, but not the contralateral eyes, showed PEX material deposits on anterior segment surfaces. Typical PEX material could be demonstrated by light and electron microscopy in the involved eyes in all known sites of PEX material deposition, such as the lens, the iris pigment epithelium, the iris stroma, the ciliary epithelium, and the trabecular meshwork. A light microscopic examination of the apparently noninvolved contralateral eyes confirmed the absence of PEX material in ocular tissues. However, by electron microscopy, subtle ultrastructural changes were found in all fellow eyes examined. All 5 eyes showed alterations of iris vessels, including basement membrane abnormalities and excessive microfibrillar deposits. In addition, minute foci of characteristic PEX fibers could be demonstrated on the iris pigment epithelium and on the unpigmented ciliary epithelium in 1 and in the area of the dilator muscle in 3 of the 5 fellow eyes.
ELECTRON MICROSCOPY

**Iris**

Whereas the iridic pigment epithelial cells of eyes with manifest PEX syndrome displayed a highly irregular basal cell surface covered by masses of PEX material, the pigment epithelial cells of contralateral eyes showed a rather regular cell surface covered by small PEX flakes in one case (Figure 1). Areas of degenerative changes of the iris pigment epithelium, such as intracellular vacuolation, swollen mitochondria, dilated rough endoplasmic reticulum, and disrupted cell membranes with liberation of melanin granules, were, however, evident in all contralateral eyes.

Degenerative alterations were also observed in the dilator muscle of contralateral eyes (Figure 2). Compared with normal eyes, the muscle cells appeared thinned and rarefied and were separated by widened intercellular spaces filled with an excess of microfibrillar material. Typical PEX fibers were found within these intercellular spaces in 3 of the 5 contralateral eyes. In some places, the muscle cells contained clumped myofilaments and degenerated cell organelles in an electronlucent vacuolar cytoplasm. Whirl-like membrane structures were found between the epithelial cells of the anterior portion of the pigment epithelium.

Compared with the control eyes, a subpopulation of iris vessels showed ultrastructural alterations in each of the 5 fellow eyes (Figure 3). These alterations included a pronounced thickening and multilayering of the endothelial basement membrane and the excessive accumulation of a fibrillar-granular extracellular material and microfibrils 10 to 12 nm in diameter in the perivascular space. Whereas mature PEX fibers were not detected, PEX fibers at different stages of maturity were observed within the perivascular matrix in 2 cases. Degenerative changes of adventitial cells, particularly pericytes, were represented by vacuolation of their cytoplasm and many pinocytotic vesicles; electron-dense extracellular granules accumulated in the vessel periphery.

**Ciliary Body**

With the exception of one contralateral eye revealing minute PEX aggregates on the surface of the unpigmented ciliary epithelium, the ciliary epithelial cells appeared normal (Figure 4). In this specific case, the epithelial cells associated with small PEX flakes displayed a regular surface outline and no ultrastructural signs of PEX fiber production. Instead, the PEX clumps seemed to be loosely adhering to the surface of the ciliary epithelium. In contrast, the unpigmented epithelial cells of obviously affected eyes were characterized by irregular invaginations of the basal cell surface containing PEX fibers. The attachment site of zonular fibers in the epithelial basement membrane of the contralateral eyes showed no alterations compared with the control eyes.

**Trabecular Meshwork**

No typical PEX deposits could be demonstrated in the trabecular meshwork or in the area of the Schlemm’s canal in any of the contralateral eyes. However, an increased amount of extracellular matrix material, including basement membrane–like and elastic material, microfibrils, collagen fibrils, and long-spacing collagen, was detected in the juxtaocular connective tissue of all contralateral eyes compared with control eyes.

**IMMUNOHISTOCHEMISTRY**

For the immunohistochemical detection of PEX-specific antigens in tissue sections of affected eyes and
contralateral eyes, antibodies against LTBP-1 were used on the light microscopic level and against the HNK-1 epitope on the electron microscopic level.

Immunofluorescent labeling with anti–LTBP-1 antibodies showed a positive immunoreaction in the periphery of a subpopulation of iris vessels and in the dilator muscle of all eyes with PEX and all contralateral eyes (Figure 5A-B and Table). Latent transforming growth factor β1–binding protein–positive deposits could also be found on the surface of the iris pigment epithelium in

Figure 2. Electron micrographs of the dilator muscle. A, Regular organization of mcs of the anterior ipe in control eyes. B, PEX deposits (star) between irregular mcs in eyes with manifest PEX syndrome. C, Widened extracellular spaces between rarefied mcs (arrows) filled with microfibrillar material and aggregates of PEX fibers (star) in a contralateral eye. D, Microfibrils and PEX fibers (star) in association with an mc in a contralateral eye. mcs indicates muscle cell process; ipe, iris pigment epithelium; and PEX, pseudoexfoliation.
eyes with PEX only. In the control eyes, a weak immuno-
reac
tion was confined to delicate fibrillar structures in the area of the dilator muscle. Furthermore, LTBP-1–
positive plaquelike deposits were detected in the con-
junctival stroma and in the periphery of conjunctival ves-
sels in eyes with PEX and in all fellow eyes (Figure 5C).

The immunogold label for HNK-1 epitopes was as-
sociated with fibrillar-granular deposits in the periph-

Figure 3. Electron micrographs of the iris vessels. A, Normal vascular en with single-layered basement membrane (arrows) and perivascular collagen fibers in control eyes. B, Pronounced multilayering of the basement membrane (arrows) and typical perivascular PEX fibrils (star) in eyes with manifest PEX syndrome. C, Abnormal fibrillar-granular extracellular material (star) and multilayered basement membrane (arrows) between en and pc in contralateral eyes. D, Sheets of basement membrane (arrows) and a thick sheath of fibrillar-granular material (star) with immature PEX fibers (arrowheads) in the perivascular space of a contralateral eye. en indicates endothelial cells; lu, lumen; PEX, pseudoxfoliation; and pc, pericytes.
ery of some iris vessels of contralateral eyes (Figure 6B). In the dilator muscle, the gold marker could be localized to electron-dense extracellular fibers, most probably representing PEX fibers (Figure 6A). The immunolocalization of the HNK-1 epitope in normal eyes and in eyes with manifest PEX syndrome corresponded to the results of previous studies and confirmed differences in composition between intraocular and extraocular PEX deposits in the conjunctiva (Table).

**COMMENT**

The present comparative study of pairs of eyes with so-called unilateral PEX syndrome demonstrated subtle PEX-specific ultrastructural changes of anterior segment tissues, particularly the iris, of all apparently not involved fellow eyes. These alterations, which were not observed in control eyes, include (1) deposits of typical PEX fibrils on the iris and ciliary epithelia and in the dilator muscle of the iris; (2) increased accumulation of extracellular matrix material, including microfibrils and basement membrane material in the periphery of iris vessels, in the dilator muscle, and in the juxtaocular connective tissue of the trabecular meshwork; and (3) degenerative changes of the iris pigment epithelium and dilator muscle tissue. Latent transforming growth factor β1–binding protein– and HNK-1–positive deposits indicating PEX material accumulations could be detected in the periphery of iris vessels and in the dilator muscle by light and electron microscopic immunolabeling in all affected and contralateral eyes, but not in the control eyes.

In addition to PEX deposits in the conjunctiva of all contralateral eyes, typical mature PEX fibers could be demonstrated on the surface of the iris and ciliary epithelia in 1 of the 5 and in the dilator muscle in 3 of the 5 contralateral eyes. These focal and rather inconspicuous PEX deposits have apparently not been visible by macroscopic and light microscopic examination of the fellow eyes. The involvement of the dilator muscle in 3 cases suggests that the iris muscles may be affected early in the
The accumulation of abnormal extracellular matrix, including PEX material, and the atrophic changes of dilator muscle cells may account for poor pupillary dilation, which is characteristic of eyes with manifest PEX syndrome but can also be observed to a minor degree in contralateral eyes. Thus, the histopathologic findings of this study indicate a bilateral, but asymmetric, compromise of the pupillomotoric function as an early manifestation of PEX syndrome.

The degenerative changes of the iris pigment epithelium in all fellow eyes may represent the morphological correlate for the well-known clinical signs characterizing early stages of PEX syndrome (melanin dispersion, peripupillary atrophy, and increased trabecular meshwork pigmentation). These pigment-related signs are observed in most of the unaffected fellow eyes.

Iris vasculopathy is a well-recognized and constant clinical feature of eyes with PEX, and involvement of blood vessel walls has been shown by many investigators. Characteristic features of this vasculopathy comprise PEX deposits in the periphery of the iris vessels; thickening and reduplication of basement membranes; and degeneration of adventitial cells explaining hypoperfusion, fluorescein leakage, and microaneurysmalizations on fluorescein angiography. Although typical perivascular PEX fibers could not be identified, abnormal accumulations of microfibrillar structures and PEX fibers at different stages of maturity were found together with basement membrane abnormalities in the periphery of a subset of iris vessels in all contralateral eyes. Comparable changes, such as basement membrane reduplications and excessive formation of microfibrils, have been described in iris vessels devoid of perivascular PEX material in eyes with manifest PEX syndrome. Furthermore, PEX fibers have been shown to be composed of and to originate from microfibrillar subunits, and elastic microfibrils have been related to the pathogenesis of PEX syndrome. In the iris stroma, PEX fibers have been reported to be associated with a fine granular matrix and to be generally shorter and thinner than PEX fibers on ocular surfaces.

### Table: Distribution of LTBP-1 and HNK-1 Positive Deposits in Normal, PEX, and Contralateral Eyes

<table>
<thead>
<tr>
<th>Eyes</th>
<th>Iris Pigment Epithelium</th>
<th>Dilator Muscle</th>
<th>Iris Vessels</th>
<th>Iris Stroma</th>
<th>Unpigmented Ciliary Epithelium</th>
<th>Trabecular Meshwork</th>
<th>Conjunctival Stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal eyes</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Contralateral eyes</td>
<td>+†</td>
<td>++</td>
<td>++</td>
<td>−</td>
<td>+†</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>Eyes with PEX</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+†</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

*LTBP-1 indicates latent transforming growth factor β1-binding protein; HNK-1, human natural killer epitope; PEX, pseudoexfoliation; −, no reaction; +, a weak positive reaction; and ++, a strong positive reaction.
†Only 2 of 5 eyes showed a positive reaction.
‡Only 3 of 5 eyes showed a positive reaction.

### Figure 6: Electron microscopic immunogold detection of the human natural killer (HNK-1) epitope in the iris of contralateral eyes.

A. Dilator muscle: association of the gold marker (arrowheads) with fibrillar-granular material in the extracellular matrix (star) between the mc. B. Iris vessels: localization of the gold marker (arrowheads) on perivascular microfibrillar deposits (star). mc indicates muscle cell processes; lu, lumen; en, endothelial cells; and pc, pericyte.
active immunolabeling of these perivascular deposits in contralateral eyes with antibodies against HNK-1 and LTBP-1 (similar to those of classic PEX) suggests that these deposits may represent early forms of PEX material. Thus, the results of the present study are in perfect agreement with the findings of a recent study17 demonstrating HNK-1–positive subendothelial deposits around the iris vessels of both eyes in patients with clinically unilateral PEX syndrome, indicating that iris vasculopathy is a constant feature of the fellow eyes.

Similarly, typical PEX accumulations could not be found in the trabecular meshwork of any of the contralateral eyes. The only alterations observed comprised an excessive accumulation of extracellular matrix material, including basement membrane–like material and microfibrillar structures in the juxtacanalicular tissue. Studies30 on aqueous humor dynamics showed a significant increase in outflow resistance in eyes with PEX compared with the fellow eyes, but also a slightly increased resistance in the fellow eyes compared with normal control eyes. Similarly, Pohjanpelto3 suggested a basic bilateral disturbance in aqueous humor dynamics leading to ocular hypertension in the fellow eyes of patients with unilateral PEX syndrome. The abnormal accumulation of extracellular materials in the critical area of the juxtacanalicular tissue could account for this increase in outflow resistance in the contralateral eyes.

The present demonstration of typical PEX fibers, microfibrillar precursors, basement membrane alterations, and degenerative cellular changes in virtually all contralateral eyes examined supports the concept that PEX syndrome is a basically bilateral disease with a clinically markedly asymmetric manifestation, as suggested by previous studies.13,17 However, the alterations are not limited to iris blood vessels but also involve the iris pigment epithelium, the dilator muscle, and the juxtacanalicular tissue of the trabecular meshwork. Minute deposits of typical PEX aggregates along the posterior chamber may even be present. Therefore, the contralateral eyes are in an early preclinical stage mainly characterized by alterations of the iris vessels, iris pigment epithelium, and dilator muscle, which precede clinically visible PEX material accumulations on ocular surfaces. The predominant iris changes might account for the clinical signs characteristic of early stages of PEX syndrome, such as melanin dispersion, peripapillary atrophy, trabecular meshwork pigmentation, poor mydriasis, and an increased protein content in the aqueous humor measured by laser tyndallometry.15,27-47 Although iris blood vessels become abnormal early in the PEX process, the clinical signs of the iris vasculopathy appear, however, to develop after the clinically visible occurrence of PEX deposits.13

Since both eyes are obviously affected by the PEX process, the term unilateral PEX syndrome, which is most common in clinical practice, is actually misleading, and the PEX syndrome is probably never truly unilateral. The reasons for this marked asymmetry remain unknown, but it may be influenced by local modulating factors, such as imbalances of growth factors, or by subtle differences in hemodynamics or aqueous humor dynamics between both eyes. In view of the wide spectrum of ocular complications associated with PEX syndrome,21 these findings should be considered in the clinical and surgical management of the patients.

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Corresponding author and reprints: Thomas Hammer, MD, Universitätsklinik und Poliklinik für Augenheilkunde der Martin-Luther-Universität Halle-Wittenberg, Magdeburger Strasse 8, 06097 Halle/Saale, Germany (e-mail: thomas.hammer@medizin.uni-halle.de).

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Archives Web Quiz Winner

Congratulations to the winner of our March quiz, Renato Ambrosio, Jr, MD, Seattle, Wash. The correct answer to our March challenge was varicella-zoster virus infection. For a complete discussion of this case, see the Clinicopathologic Reports section in the April ARCHIVES (Greven CM, Singh T, Stanton CA, Martin TJ). Optic chiasm, optic nerve, and retinal involvement secondary to varicella-zoster virus. Arch Ophthalmol. 2001;119:608-610).

Be sure to visit the Archives of Ophthalmology World Wide Web site (http://www.archophthalmol.com) and try your hand at our Clinical Challenge Interactive Quiz. We invite visitors to make a diagnosis based on selected information from a case report or other feature scheduled to be published in the following month's print edition of the ARCHIVES. The first visitor to e-mail our Web editors with the correct answer will be recognized in the print journal and on our Web site and will also receive a free copy of the book One Hundred Years of JAMA Landmark Articles.