Autopsy Analysis of Clinically Unilateral Exfoliation Syndrome

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Purpose. To study the pathogenesis of clinically unilateral exfoliation syndrome by localizing exfoliation deposits in involved and fellow eyes during autopsy.

Methods. The formalin-fixed and paraffin-embedded involved and fellow eyes were obtained at autopsy from five patients (age range, 72 to 88 years) with clinically unilateral exfoliation. Exfoliation deposits were identified with monoclonal antibodies (mAb) HNK-1 and NC-1 to the HNK-1 carbohydrate epitope, and with five lectins (Bauhinia purpurea agglutinin, Concanavalin A, Lens culinaris agglutinin, Phaseolus vulgaris erythroagglutinin, and Ricinus communis agglutinin I) using the avidin-biotinylated peroxidase complex (ABC) method.

Results. Marked exfoliation deposits in all involved eyes, and weak exfoliation deposits in one fellow eye were consistently detected in light microscopic, immunohistochemical, and lectin histochemical examinations. Similarly labeled deposits were present around a population of blood vessels of the iris in every involved and fellow eye. Particularly in fellow eyes, these subendothelial deposits were better visualized with mAbs to the HNK-1 epitope than they were with lectins. In the only fellow eye with early exfoliation, the reactivity around blood vessels was more conspicuous than the exfoliation deposits, whereas the reverse was true in the involved eyes.

Conclusions. Clinically unilateral exfoliation is asymmetric, rather than truly monocular. The findings in fellow eyes suggest that iris blood vessels become abnormal early in the process, even before exfoliation deposits are histopathologically seen in the posterior chamber. Marked asymmetry in exfoliation indicates an influence of modulating local factors that may be internal or external to the eye, and that also may be functional in bilateral exfoliation.


Exfoliation syndrome is characterized by whitish material of unknown composition and origin that gradually accumulates in the posterior and anterior chamber on the free surfaces of the ciliary epithelium, zonules, hyaloid face, anterior lens capsule, iris, corneal endothelium, and trabecular meshwork, and within the iris stroma.1,2 Fibrils that resemble those of exfoliation material have been found in electron microscopic examination of several extraocular tissues.3-10 Although exfoliation eventually tends to affect both eyes, as would be expected of a systemic disorder, up to two thirds of patients have deposits in one eye only.11-15 Occasionally this unilaterality persists until advanced age.

Studies that focus on seemingly unilateral exfoliation are surprisingly rare,3,16-25 but their results show that the involved eye has a reduced outflow facility,16 more pigment in the chamber angle,12,24 a higher intraocular pressure,13,16-19,21,22 more evidence of disc damage,22 poorer visual acuity,21,23 and more advanced lens opacity12,15,21,23 than does the fellow eye. With the exception of ultrastructural studies of conjunctiva,4 the intriguing problem of monocular exfoliation has not received the attention of pathologists; yet it might well shed light on the unresolved pathogenesis of exfoliation in general.

To study the histopathology of unilateral exfoliation, we examined five pairs of eyes taken during autopsy from patients in whom heavy exfoliation in one eye had recently been diagnosed, with no exfoliation in the fellow eye. These eyes were analyzed with two
TABLE 1. Characteristics of the Eyes With Clinically Unilateral Exfoliation Deposits

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Clinically Detected Exfoliation Deposits</th>
<th>Intercurrent Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right Eye</td>
<td>Left Eye</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>72</td>
<td>Heavy</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>75</td>
<td>None</td>
<td>Heavy</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>78</td>
<td>Heavy</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>82</td>
<td>Heavy</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>88</td>
<td>None*</td>
<td>Heavy</td>
</tr>
</tbody>
</table>

* Weak by histopathology.

Monoclonal antibodies (mAb) to the HNK-1 carbohydrate epitope, which is a marker for exfoliation material,²⁵,²⁶ as well as with five lectins that have proved to be useful in identifying exfoliation deposits.¹⁰,²⁷⁻³⁰

MATERIAL AND METHODS

Histologic Specimens

Five consecutive pairs of formalin-fixed, paraffin-embedded eyes (Table 1) obtained during autopsy within 1 to 3 days after death from patients with clinically unilateral exfoliation syndrome were collected from the files of the Ophthalmic Pathology Laboratory, Department of Ophthalmology, Helsinki University Central Hospital. Each patient’s chart indicated that, in biomicroscopic studies made through dilated pupils, marked exfoliation deposits had been noted in one eye, whereas no deposits had been seen in the fellow eye (Table 1). The eyes had been studied during autopsy because of a history of prior ocular disease or surgery (Table 1). Tenets of the Declaration of Helsinki were followed in the study.

The phakic eyes were sectioned horizontally and the pseudophakic ones at the equator. Macroscopic exfoliation deposits were searched for using a high-powered dissecting microscope (Olympus SZH, Olympus Optical, Tokyo, Japan). If an intraocular lens was present, the posterior capsule and the haptics of the lens were cut, the optic of the intraocular lens was removed, and the anterior segment was sectioned vertically before processing for embedding.

Sections (5-μm thick) were cut from the specimens and mounted on chromium–gelatin-treated glass slides to ensure tissue adherence (0.05 g potassium chromate III sulfate dodecahydrate and 0.5 g gelatin in 100 ml of distilled water). The routine histopathologic stains were hematoxylin–eosin, van Gieson, and periodic acid–Schiff.

Immunohistochemical Staining

Two mouse monoclonal immunoglobulin M antibodies (mAbs) to the HNK-1 carbohydrate epitope, HNK-1 (Leu-7, lot 30015, Becton Dickinson, San Jose, CA; diluted 1:40)³¹ and NC-1 (CD57, lot 2204-01-02, Central laboratorium van de bloedtransfusiedienst, Amsterdam, The Netherlands; diluted 1:25)³² were obtained commercially. An unrelated immunoglobulin M mAb to cytokeratin 14 (CKB1, lot 100H4800, Sigma St. Louis, MO; diluted 1:300) was used as a negative control.

The immunoperoxidase staining was done using a commercial version (Vectastain ABC Elite Kit for Mouse IgG, Vector Laboratories, Burlingame, CA) of the avidin-biotinylated peroxidase complex (ABC) method and 3-amino-9-ethylcarbazole as chromogen, as has been described previously in detail.²² Omission of the primary antibody, the secondary antibody, or the ABC resulted in loss of all immunoreaction.

<table>
<thead>
<tr>
<th>Plant of Origin</th>
<th>Nominal Carbohydrate Specificity</th>
<th>Inhibitor</th>
<th>Binding to Exfoliation†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bauhinia purpurea alba (BPA)</td>
<td>α/βDGalNAc &gt; α/βDGal</td>
<td>dGal</td>
<td>Strong</td>
</tr>
<tr>
<td>Canavalia ensiformis (ConA)</td>
<td>αMan &gt; αDGlc</td>
<td>αMetDMan</td>
<td>Strong</td>
</tr>
<tr>
<td>Lens culinaris (LCA)</td>
<td>αMan &gt; αDGlc</td>
<td>αMetDMan</td>
<td>Strong</td>
</tr>
<tr>
<td>Phaseolus vulgaris (PHA-E)</td>
<td>DGal(β1 → 4)DGlcNAc(β1 → 2)DMAn</td>
<td>Fetuin</td>
<td>Strong</td>
</tr>
<tr>
<td>Ricinus communis (RCA-I)</td>
<td>βDGal &gt; αDGal ≡ αDGalNAc</td>
<td>Lactose</td>
<td>Strong</td>
</tr>
</tbody>
</table>

Met = methyl; Gal = galactose; Glc = glucose; Man = mannose; GalNAc = N-acetylgalactosamine; GlcNAc = N-acetylgalcosamine.

* Data from Goldstein and Poretz.³³
† Data from Hietanen and Tarkkanen²⁷ and Hietanen et al.²⁹
Lectin Histochemistry

Agglutinins (Table 2) from Bauhinia purpurea alba (lot 27F-4021), Canavalia ensiformis (Concanavalin A, lot 49F-8030), Lens culinaris (lot 61H-3903), Phaseolus vulgaris (erythroagglutinin, PHA-E, Type III-B, lot 77F-4012) and Ricinus communis (agglutinin I, RCA-120, lot 20H-4051) conjugated to biotin were commercially obtained (Sigma), diluted with phosphate-buffered saline (PBS, pH 7) to a protein concentration of 500 μg/ml, and stored at −20°C until needed. When used, they were further diluted to a concentration of 100 μg/ml.

The lectin histochemical staining was done using a commercial version (Vectastain ABC Elite Kit, Vector Laboratories) of the ABC method, as described earlier. The sections were pretreated with pepsin to enhance the availability of lectin-binding sites in formalin-fixed, paraffin-embedded material. Parallel negative control sections were stained after preincubating the lectins with their corresponding hapten sugar inhibitors (Table 2).

RESULTS

Under a dissecting microscope, all five clinically involved eyes showed typical exfoliation deposits on the
Unilateral Exfoliation

Antibodies to the HNK-1 epitope intensely labeled exfoliation deposits in all involved eyes (Fig. 2A) on the ciliary epithelium (Fig. 1G), on the lens capsule (Fig. 1I), and on the epithelium of the iris. Heavy exfoliation was detected in four involved eyes and moderate in the fifth one. In spite of the heavy exfoliation, no labeling of the corneal endothelium or keratocytes was detected in any of the involved eyes. The five lectins used also strongly labeled the exfoliation deposits, but the reaction intensity with *Lens culinaris* was somewhat weaker than with the other lectins (Fig. 2A).

In all fellow eyes, antibodies to the HNK-1 epitope labeled the basement membrane of the nonpigmented ciliary epithelium (Fig. 1H) that also bound the five lectins used. No unequivocal deposits consistent with exfoliation material were revealed in four fellow eyes (Fig. 2B). In the fifth eye, in which weak exfoliation was seen in light microscopic study, weak deposits on the iris epithelium were confirmed, with both antibodies to the HNK-1 epitope and with the five lectins (Fig. 2B).

Iris Stroma and Blood Vessels

Both antibodies to the HNK-1 epitope consistently bound to the subendothelial region of a population of iris blood vessels in all involved eyes (Fig. 3A). A similar perivascular labeling that was less extensive was regularly found in all fellow eyes (Fig. 3B). The granular pattern of the immunoreaction formed a thick, uniform layer beneath the vascular endothelium (Figs. 3A to 3C). The immunoreaction involved the zone of the thick, laminated basement membrane and the inner part of the loose collagenous zone of iris blood vessels (Figs. 3A to 3C). However, in involved (Fig. 3C) and fellow eyes, the intensity of the immunoreaction varied widely from vessel to vessel, and a notable number of vessels remained essentially unlabeled, even in involved eyes.

In addition, many small immunopositive deposits, apparently unassociated with blood vessels, were present within the stroma of the iris in the involved eyes, particularly close to the anterior border layer (Figs. 1A to F; periodic acid–Schiff) and immunohistochemical (G to J) analysis of exfoliation deposits in the involved (left column) and corresponding fellow (right column) eyes in clinically unilateral exfoliation syndrome. (A) In an involved eye, the iris epithelium has an irregularly serrated contour, is flattened, and is covered by a layer of exfoliation deposits, whereas (B) in the corresponding fellow eye, the epithelium is normal in thickness, regularly serrated, and without exfoliation deposits. Note that stromal collars of blood vessels (arrowheads) are moderately periodic-acid–Schiff-positive in the involved eye. (C) In one patient, the involved iris epithelium is covered by a thick layer of partly bush-like exfoliation deposits, and (D) weak deposits (arrowheads) are additionally found in the fellow eye. (E) In the ciliary sulcus, exfoliation deposits are prominent in an involved eye, whereas (F) no deposits are seen in the fellow eye. (G) Monoclonal antibody NC-1 to the HNK-1 epitope labels thick layers of exfoliation on the surface of the nonpigmented ciliary epithelium in an involved eye, whereas (H) only its basement membrane (arrowheads) is immunoreactive in the fellow eye. (I) Monoclonal antibody NC-1 likewise reacts with heavy exfoliation deposits on the surface of the lens capsule and in the zonular lamella (arrowheads) in an involved eye, but (J) labels only the normal zonular lamella (arrowheads) in the fellow eye.

ce = ciliary epithelium cs = ciliary sulcus; ex = exfoliation deposits; ie = iris epithelium; le = lens. Original magnifications, (A,B) ×210; (C to J) ×420.

zonaules and on the ciliary processes, and the iris epithelium was characteristically irregularly serrated. No exfoliation deposits were noted in four of the five fellow eyes. Trace exfoliation was suspected to be present in the fifth eye (Table 1).

Light microscopic examination confirmed the presence of heavy exfoliation in the posterior chamber and marked degeneration of the iris epithelium in all involved eyes, compared with that noted in the fellow eyes (Figs. 1A to 1F), as well as the complete absence of such deposits on the surface of the iris (Figs. 1A, 1B, 1E, 1F), the nonpigmented ciliary epithelium (Figs. 1G, 1H), and the lens capsule (Figs. 1I, 1J) in four fellow eyes, compared with the presence of the deposits in those areas of the corresponding involved eyes. In particular, no deposits were seen in the ciliary sulcus of the fellow eyes, whereas prominent deposits were present in all involved eyes (Figs. 1E, 1F).

Weak deposits along the iris epithelium (Fig. 1D) were noted in the only fellow eye in which macroscopic examination revealed trace exfoliation. The deposits were minor, however, compared with the particularly heavy exfoliation diagnosed in the corresponding involved eye (Fig. 1C).

Immunohistochemistry and Lectin Histochemistry

The immunohistochemical and lectin histochemical findings in the involved eyes and fellow eyes are summarized in Figure 2.

Anterior Chamber

Antibodies to the HNK-1 epitope intensely labeled exfoliation deposits in all involved eyes (Fig. 2A) on the ciliary epithelium (Fig. 1G), on the lens capsule (Fig. 1I), and on the epithelium of the iris. Heavy exfoliation was detected in four involved eyes and moderate
FIGURE 2. Presence of exfoliation deposits along the posterior chamber of (A) involved eyes and (B) of fellow eyes, and of subendothelial immunoreaction around blood vessels of the iris in (C) involved eyes and (D) in fellow eyes in the five patients studied, identified with mAb HNK-1 and NC-1 to the HNK-1 epitope and with five lectins (for abbreviations, see Table 2). Weak exfoliation deposits were identified in one fellow eye; all deposits had a vasculopathy that was similar to the one observed in the involved eyes.

3C, 3D). Identical perivascular and stromal deposits were revealed with the five lectins used (Figs. 3E to 3H), although the slides were more difficult to interpret because of additional labeling of normal stromal elements (Figs. 3E, 3F), particularly in staining with Concanavalin A, Phaseolus vulgaris-erythroagglutinin, and Ricinus communis agglutinin. Regarding the subendothelial deposits, all lectins and antibodies to the HNK-1 epitope gave essentially identical labeling patterns when adjacent sections were stained (Figs. 3G, 3H).

The subendothelial perivascular immunoreaction was heavy in one involved eye and moderate in four (Fig. 2C), although the reaction was more variable in intensity than was labeling of the classic exfoliation deposits in the posterior chamber. In fellow eyes, the subendothelial vascular deposits were generally weak (Fig. 2D), but always easily detectable, in particular with mAb NC-1 to the HNK-1 epitope, which was the most sensitive reagent for detecting the subendothelial deposits.

DISCUSSION

Consistent with clinically unilateral exfoliation syndrome in the eyes of the five patients studied, routine examination under light microscope revealed markedly asymmetric exfoliation deposits. Indeed, no evidence of even weak exfoliation in the posterior chamber was detected in four fellow eyes. The apparent unilaterality was further verified in these patients with two antibodies to the HNK-1 epitope and with five lectins that strongly react with exfoliation material. In the fellow eye of the fifth and oldest patient in the series, early deposition of exfoliation material on the epithelium of the iris was detected. Even in this patient, the syndrome was remarkably asymmetric.

Whereas results of immunohistochemical and lectin histochemical studies were mainly of confirmatory value in localizing exfoliation deposits in the posterior chamber, they added significantly to elucidation of the vasculopathy that is known to be present in the iris of eyes with exfoliation but that is not readily seen without electron microscopy. Subendothelial labeling identical to that of classic exfoliation material was regularly detected in the iris, not only in all involved eyes, but also in every fellow eye. In accordance with results of ultrastructural studies, these apparent exfoliation deposits occurred only in a population of iris blood vessels, and they were unevenly distributed. The vasculopathy was more advanced in involved eyes, in which similarly labeled stromal deposits were also highlighted by lectin and immunohistochemistry, reflecting the severity of exfoliation syndrome in them. Such stromal deposits have previously
FIGURE 3. Immunohistochemical (A to D,G) and lectin histochemical (E,F,H) analysis of exfoliation-related vasculopathy in irides of involved (A,C to E,G,H) and corresponding fellow (B,F) eyes in clinically unilateral exfoliation syndrome. (A) In an involved eye, mAb NC-1 to the HNK-1 epitope reveals a thick subendothelial layer of granular immunoreaction around blood vessels of the iris. (B) In the fellow eye, a similar but less pronounced immunoreaction is present. Note that the immunoreaction involves the thick laminated basement membrane and part of the loose collagenous zone of the vessel wall, but the dense collagenous zone (delimited by arrowheads) is unlabeled. (C) In an involved eye, the subendothelial immunoreaction varies from strong (arrow) to weak (arrowheads). Note a stromal deposit (double arrowhead) adjacent to a vessel. (D) Similar immunopositive deposits (arrowheads) are present at the anterior border layer of the iris along the anterior chamber and within the stroma, apparently unassociated with vessels. (E) In an involved eye, the subendothelial layer of many iris vessels also strongly binds *Phaseolus vulgaris* erythroagglutinin. Only the endothelium (arrow) of other vessels is labeled. (F) In a fellow eye, the subendothelial layer variably and weakly binds *Bauhinia purpurea* agglutinin. Note that both lectins also bind to stromal cells (arrowheads). (G) Monoclonal antibody NC-1 and (H) *Bauhinia purpurea* agglutinin identically label the subendothelial layer of an involved eye in adjacent sections. ac = anterior chamber; se = subendothelial layer; str = stroma. Original magnifications, (A,B) X420; (C) X210; (D to H) X420.
been seen in electron microscopic examination of eyes with exfoliation syndrome.27,30,40

An identical vasculopathy has been noted previously with antibodies to the HNK-1 epitope in all studied eyes with exfoliation syndrome, as well as in approximately one third of eyes of patients without exfoliation deposits visible in light microscopic examination.25 Such an immunoreactivity was more common in older than in younger age groups. Because exfoliation syndrome develops with age, the evidence supports the theory that the vasculopathy may represent an early preclinical stage of this syndrome and that it is an integral part of the disease. Whether the vasculopathy is causally related to development of classic exfoliation deposits in the posterior chamber is not yet known, however. Nevertheless, we have shown conclusively that vasculopathy is detectable earlier than are frank exfoliation deposits on the ciliary epithelium and lens capsule.

How is strikingly asymmetric exfoliation to be explained if the fibrillopathy seen by electron microscopy is a systemic condition?8–10 It appears obvious that one or more local factors, either internal or external to the eye, must be invoked to expedite deposition of exfoliation material in one eye or slow it down in the other. The eye in which classic exfoliation deposits form may be compromised in some way—for example, in the rate of aqueous flow,16,19 in the arterial circulation,20,41 or in some other as yet unidentified factor.

In the current series, a cataract necessitating surgery, glaucoma, and vascular accidents had been present in all involved eyes, whereas no complications traditionally related to exfoliation had occurred in the fellow eyes, as is common in clinical practice.1,12,14,20,42 We do not know for sure whether they are logical sequelae of exfoliation12,14,17,20 or are parallel findings15,19 to which as yet unknown factors also leading to clinical exfoliation contribute in a compromised eye.

Even though most investigators currently appear to hold the view that exfoliation syndrome results from excess production or hyperactive proteolysis of basement membrane components, elastic fibers, or zonules,37,43–46 it is good to keep in mind that in most storage diseases as well as in amyloidosis, tissue deposition is ultimately related to a failure to degrade normally or to clear the precursor molecules.47 In particular, the iris vasculopathy in exfoliation syndrome might be either a cause for or a consequence of failed clearance from the eye of material that ultimately aggregates as exfoliation deposits. For example, although it has been proposed that exfoliation material is produced in the iris,50,45 the vasculopathy might alternatively be secondary to decreased solubility25 or increased adhesiveness25 of abnormally processed intraocular macromolecules.

In summary, exfoliation deposits can be markedly asymmetric to the extent that they appear to be unilateral, even in routine histopathologic evaluation. Secondly, a vasculopathy characterized by subendothelial deposits that react identically to exfoliation material is universal in the fellow eyes and also is common in eyes of older people without visible classic exfoliation deposits.25 Such vasculopathy apparently heralds clinical exfoliation and is more advanced in eyes with visible exfoliation deposits. Thus, although frequently asymmetric, exfoliation probably is never truly unilateral. Obviously, any theory of exfoliation should include explanation of the lack of exfoliation deposits in the face of a systemic fibrillopathy, the common occurrence of asymmetric exfoliation, and the early, scattered vasculopathy. In the future, we should probably concentrate our efforts on understanding why and how exfoliation deposits form, rather than solely on what they contain.

Key Words
carbohydrates, iris, lectins, leu-7, pseudoexfoliation

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References