The value of conjunctival biopsy in childhood cystinosis

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Summary. Cystinosis is frequently presented with cystine storage in the cornea and conjunctiva, and the diagnosis can be established by slit-lamp examination. It can also be confirmed by electron microscopy of a conjunctival biopsy.

The present paper reports on a 16-month-old boy with Fanconi’s syndrome, in whom the slit-lamp examination did not show crystal deposits of cystine in the conjunctiva. The ultrastructural study of the conjunctival biopsy demonstrated polygonal crystals within double membrane-limited organelles located in fibroblasts. Similar crystals were subsequently found in a kidney biopsy. We therefore think that conjunctival biopsy is a valuable diagnostic tool prior to performing renal biopsy, even in cases with negative findings by ophthalmologic examination.

Key words: Cystinosis, Ultrastructure, Conjunctival biopsy, Lysosomes

Introduction

Cystinosis is a recessively inherited metabolic disorder characterized by intracellular deposition of cystine crystals in eye, kidney, reticuloendothelial system and other tissues (Schneider et al., 1978). The underlying biochemical defect remains unidentified, but is believed to be a lysosomal disorder (Schulman et al., 1970; Wong et al., 1970; Kenyon and Sensenbrenner, 1974).

In general, three types of cystinosis are distinguished: infantile, adolescent, and adult cystinosis (Dodd et al., 1978). The former is the most severe type: it is a common cause of Fanconi’s syndrome and progressive renal glomerular damage and leads to death within the first decade of life (Koizumi et al., 1985).

All three types of cystinosis are frequently presented with crystalline cystine storage in the cornea and conjunctiva, and the diagnosis can be established by slit-lamp examination (Stefani and Vogel, 1982). The conjunctival ultrastructure has been studied in detail in cystinotic patients, but to our knowledge, a preliminary diagnosis was previously established by slit-lamp biomicroscopy in all cases (Kenyon and Sensenbrenner, 1974; Levine and Paparo, 1982; Stefani and Vogel, 1982; Dodd et al., 1987).

In this paper, we report on a 16-month-old boy who had been diagnosed having Fanconi’s syndrome. The clinical ocular findings were negative and the ophthalmological examination including slit-lamp observation did not demonstrate crystalline deposits.

Materials and methods

Case report

The patient was a 16-month-old boy. Pregnancy and birth were uncomplicated. No abnormalities were noted in the neonatal period. He had received the usual preventive doses of vitamin D (400 units/day). The patient was hospitalized because of polyuria, polydipsia and gait disturbance. Examination on admission revealed a pale boy with hypopigmentation of the skin and hair, dehydration, marked growth retardation, rachitic rosary, and Harrison’s groove. He had cervical, axillary, and inguinal lymphadenopathy and also mild hepatomegaly. Laboratory tests demonstrated metabolic acidosis, hypokalemia, hypophosphatemia, increased blood pyruvate concentration, glucosuria, aminoaciduria, phosphaturia, and proteinuria. Microscopic examination of urine showed occasional erythrocytes and abundant leukocytes. Proteus mirabilis was demonstrated by urine culture. A complete blood cell count was normal. Ultrasonography and intravenous pyelography showed an enlarged right...
Conjunctival biopsy in cystinosis

kidney with mildly dilated pelvis and calyces, in which multiple round stones were found. The left kidney was normal. The ophthalmologic examination, including slit-lamp observation and indirect ophthalmoscopy, was unremarkable. Despite the negative ocular findings a biopsy was taken from the bulbar conjunctiva.

One month later, the patient underwent right nephrectomy.

Technical procedure

Conjunctival and renal tissues were processed as follows: fresh tissue specimens were fixed in Karnowski's solution, post-fixed with 1% OsO₄ in phosphate buffer, and embedded in Araldite M. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined using a Zeiss EM 10 microscope.

Some fragments of the renal specimen were also fixed in 10% formaldehyde, and paraffin sections were processed with conventional histological techniques.

Results

The electron-micrographs of the conjunctiva showed polygonal crystalline species filled with fine granular material located in fibroblasts. These crystals appeared within double membrane-limited organelles that frequently displayed the margined electron-dense material characteristic for secondary lysosomes (Fig. 1), which were visible at higher magnification. In one area, empty spaces of crystalline appearance were seen between collagen bundles of variable size and spheric shape. Myelin-like bodies and vesicular membrane structures (Fig. 2) were also found in some crystals.

Light microscopy of the kidney revealed few crystals in epithelial tubular cells and also histiocytic and interstitial foam cells. The tubules showed scattered dilatation of the proximal and distal portions. There was a prominent diffuse infiltrate of leukocytes in the interstitium. No glomerular changes were seen.

Electron microscopy of the renal tissue showed

Fig. 1. Conjunctival biopsy. Crystals with double membrane-limited organelle displaying the margined electron-dense material in secondary lysosomes. × 12,500, inset × 25,000
polygonal crystals (Fig. 3) mainly in the interstitial histiocytes but also in epithelial tubular cells. The morphological appearance of these crystals was identical to that of the conjunctival ones. Ultrastructural appearance of the glomeruli was unremarkable.

Discussion

Our 16-month-old patient showed the clinical features of Fanconi's syndrome. The causes of renal Fanconi's syndrome include a variety of hereditary and acquired disorders (Schneider et al., 1978). Cystinosis is a common cause of this syndrome in children. The diagnosis is established by demonstrating the presence of cystine crystals in the cornea and conjunctiva by slit-lamp examination. Additional ocular findings include patchy depigmentation of the macula (Dodd et al., 1978).

In the present case, the slit-lamp examination failed to demonstrate cystine crystals. These negative findings may be explained by the short time of evolution. Indirect ophthalmoscopy showed no retinal depigmentation. To our knowledge, the absence of retinopathy in the childhood form has so far only been reported in one autopsy case (Koizumi et al., 1985).

The morphologic appearance and location of conjunctival crystals in the present case were similar to those reported previously (Kenyon and Sensenbrenner, 1974; Dodd et al., 1978), except for the unusual presence of
myelin-like bodies and vesicular membrane structures. The latter could be cystine accumulations.

Light and electron microscopy of our patient's kidney specimen are in agreement with other studies (Schneider et al., 1978; Koizumi et al., 1985), and the electron microscopy characteristics of crystals in the conjunctiva and kidney were likewise similar.

This case illustrates the importance of conjunctival biopsy as a diagnostic tool prior to renal biopsy in children with Fanconi's syndrome, even with negative findings by indirect ophthalmoscopy and slit-lamp examination.

**Fig. 3.** Renal biopsy. Polygonal crystals in interstitial histiocytes. × 5,000, inset × 60,000

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**References**


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