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Ultrastructure of Connective Tissue of Eye Drainage System in Ophthalmic Hypertension Associated with Primary Juvenile Glaucoma

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In primary juvenile glaucoma, the connective tissue of the juxtacanalicular zone and sclera undergo constant reorganization because of incessant synthesis and degradation of extracellular matrix components with pronounced fluctuations of electron density of collagen fibrils. Destructive changes in the perivascular nerve trunks were detected at all stages of the glaucoma process, including the initial stages: numerous large foci of cytoplasmic organelle destruction with the formation of autophagosomes and residual bodies predominated in the cytoplasm of myelinated axons of nerve cells.

Key Words: *primary juvenile glaucoma; connective tissue; electron microscopy*

Glaucoma is one of the most important problems of ophthalmopathology. Many-year studies of one of its most prevalent forms, primary open-angle glaucoma, detected the main constituents in the pathogenesis, but no universal concept was formulated [4,6]. All morphological studies of various glaucoma forms were focused on the drainage system of the eye, mainly on its two structural constituents: trabecular network and endothelium of Schlemm's canal [8-12].

Primary juvenile glaucoma (PJG) is regarded as a heterogeneous multifactorial disease; its development are explained by genetic predisposition with "accumulation" of diseases associated with connective tissue dysplasia in the families [5,7]. For example, significant changes in qualitative and quantitative composition of glycosaminoglycans of the trabecular networks of the eye and intervertebral disc were demonstrated in PJG [1,2].

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We analyzed connective tissue components of the juxtacanalicular zone and sclera in PJG at the ultrastructural level.

MATERIALS AND METHODS

Operation material obtained by nonpenetrating deep sclerectomy (specimens of the sclera, juxtacanalicular tissue, and outer wall of Schlemm's canal) from 55 patients with glaucoma was analyzed. The patients formed 2 groups: 1) PJG of different stages (n=25: 13 with initial and developed stages, 8 with advanced, and 4 with the terminal stage) and 2) primary open-angle glaucoma (n=30, 10 per glaucoma stage). Group 1 consisted of 13 male and 12 female patients aged 11-35 years (mean age 24.8± 4.6 years) and group 2 of 21 men and 9 women aged 50-78 years (mean age 63.8± 14.2 years).

The material was fixed in 4% paraformaldehyde and examined under light and electron microscopes. Paraffin sections were stained with hema-

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toxylin and eosin in combination of Pearls' reaction, after Van-Gieson with poststaining of elastic fibers with Weigert's resorcin-fuchsin, and PAS reaction was carried out. Semithin sections were stained with Schiff's reagent and 1% azur II. Lihgt microscopy was carried out under a universal Leica DM 4000B microscope with Leica DM 320 digital cam. Ultrathin sections contrasted saturated ethanol solution of uranylacetate and lead citrate were examined under a JEM 1010 electron microscope at accelerating voltage of 80 kV.

RESULTS

Morphological study of specimens of the operation material from patients with PJG revealed a sort of a staged pattern in the structural changes of drainage system components, largely corresponding to the severity of optic nerve atrophy and impairment of visual functions.

The zone of the juxtacanalicular tissue adjacent to the outer wall of Schlemm's canal thinned with PJG progress: the minimum count of cells and nar-

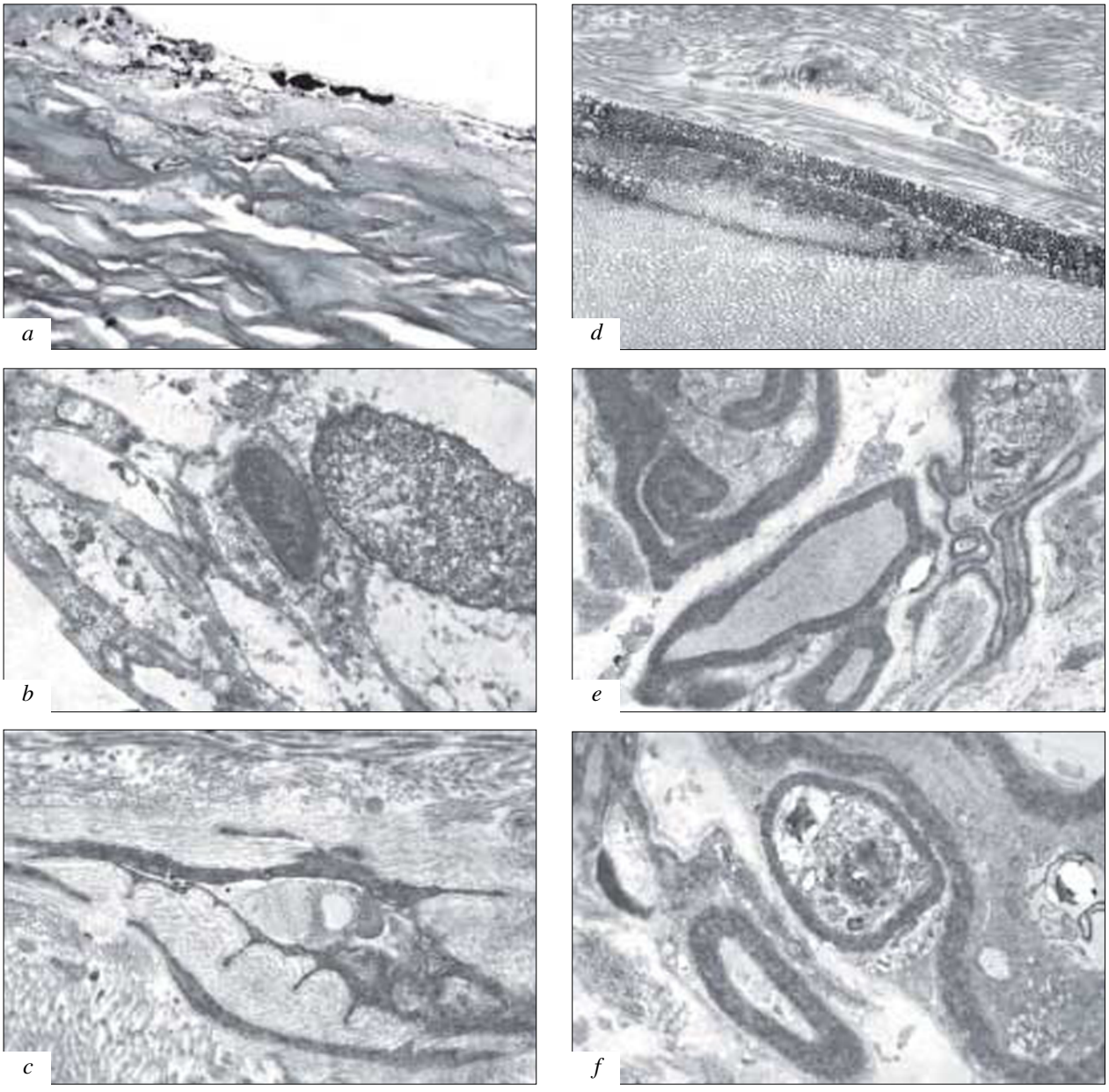

Fig. 1. Structural characteristics of connective tissue and nerve components of the eye in PJG. *a*) paraffin section, PAS reaction; *b-f*) electronograms. *a*) juxtacanalicular zone atrophy, ×650; *b*) degradation of juxtacanalicular zone cytoplasmic organelles of the cell, ×8000; *c*) heterogeneity of connective tissue fibrous components, ×8000; *d*) sharp fluctuations of osmiophilia of scleral collagen fibrils, ×6000; *e*, *f*) autophagosome formation in myelin nerve fibers, disorganization of the nerve bundle connective tissue structures: *e*: ×6000, *f*: ×8000.

rowing of the fibrous component zone (with predominating thick bundles of collagen fibers with signs of hyalinosis) were seen. Remaining elastic fibers underwent destruction (fragmentation and changes of typical tinctorial characteristics). The terminal stage of PJG was associated with pronounced destruction and atrophy of the juxtacanalicular tissue (Fig. 1, *a*).

Connective tissue cells in the juxtacanalicular zone were discernible only at the initial stages of the glaucomatous process and exhibited ultrastructural signs of the cytoplasmic organelle degradation (Fig. 1, *b*); collagen fibers varied significantly by thickness, electron density, and striation pattern at all stages of PJG development, forming conglomerations with elastic fibers, whose amorphous component showed a trend to increase of osmiophilia as glaucoma progressed (Fig. 1, *c*).

Scleral connective tissue at the initial stages of PJG was characterized by dyscomposition (loosening of fibers) and focal metachromatic staining. The density of fibrous structures increased with enlargement of the metachromatic staining zone at advanced and terminal stages in comparison with the initial stages of glaucomatous process, this indicating intensive disorganization of the connective tissue. Electron density of collagen fibrils in the deep layers of the sclera sharply fluctuated (Fig. 1, *d*).

Significant changes in the qualitative and quantitative composition of glycosaminoglycans were detected in PJG presenting as a trend to reduction of the content of sulfated glycosaminoglycans, diffuse proteoglycans, and a significant increase in the content of collagen-bound proteoglycans, indicating collagenogenesis intensification in the juxtacanalicular tissue and deep layers of the sclera [1], which was confirmed by the results of ultrastructural analysis of the operation material.

Destructive changes in axons of perivascular nerve trunks in the sclera were detected at all, even initial stages of the glaucomatous process. Numerous large foci of destruction of cytoplasmic organelles with the formation of heterogeneous autophagosomes and myelin figures predominated in the cytoplasm of myelinated axons; the rest part of the cytoplasm retained solitary small mitochondria with solitary cristae (Fig. 1, *e*, *f*). It seems that incompetence of the trophic function of connective tissue cells plays a certain role in destructive changes in neurons of nerve bundles, the ultrastructure of these cells attests to systemic type of degenerative changes.

Metabolism and catabolism of the juxtacanalicular and scleral connective tissue were less intense in primary open-angle glaucoma in comparison with juvenile glaucoma; pigment imbibition of Schlemm's canal wall and mononuclear cell infiltration were more pronounced.

Hence, light microscopy and ultrastructural analysis showed heterogeneous structural changes of different degree in the connective tissue components of the drainage system of the eye. The juxtacanalicular tissue adjacent to the endothelium at first exhibited a trend to more intensive fibrogenesis followed by connective tissue degradation: changes in the tinctorial characteristics of collagen and elastic fibers, cell element reduction, leading to thinning of this layer. Ultrastructural analysis showed heterogeneity of collagen fibrils with a trend to increase of their osmiophilia with the disease progress.

The findings of structural and biochemical analysis can be interpreted from the standpoint of the concept on relationship of desmolytic and desmoplastic functions [3]. Abnormal restructuring of connective tissue determines diffuse degenerative atrophic changes in the ocular drainage system and intrascleral nerve fibers and underlies all subsequent structural and functional changes in the ocular drainage system.

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