Clinical and Ocular Histopathological Findings in a Patient With Normal-Pressure Glaucoma

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Objective: To study the histopathological changes of eyes from a patient with normal-pressure glaucoma whose clinical and laboratory findings were well documented.

Methods: Postmortem histopathological findings in a patient with normal-pressure glaucoma who had monoclonal gammopathy and serum immunoreactivity to retinal proteins were examined in comparison with those of an age-matched control subject. Clinicopathological correlations to laboratory findings were examined.

Results: Clinical and histopathological findings of the patient, including cavernous degeneration of optic nerve and characteristic optic nerve cupping, were similar to those in patients with glaucoma who had high intraocular pressure. In addition, we found immunoglobulin G and immunoglobulin A deposition in the ganglion cells, inner and outer nuclear layers of the retina, and evidence of apoptotic retinal cell death using terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling technique.

Conclusions: Serum antibodies to retinal proteins and retinal immunoglobulin deposition constitute novel findings in a patient with normal-pressure glaucoma and may contribute to better understanding of the mechanisms underlying glaucomatous optic neuropathy in this disorder.


GLAUCOMATOUS optic neuropathy is characterized by loss of retinal ganglion cells and their axons, excavated appearance of optic nerve head, and progressive loss of visual field sensitivity. Although clinical studies have shown the role of several risk factors in glaucomatous optic neuropathy, including high intraocular pressure (IOP), about 20% to 25% of glaucomatous optic neuropathy develops in patients with normal IOP. Despite several histopathological reports of postmortem human eyes from patients with primary open-angle glaucoma, and despite experimental studies using glaucoma models in which IOP is elevated, it is not clear whether the pathological findings of glaucomatous eyes with normal IOP are similar to those seen in glaucomatous eyes with high IOP. We herein present the clinical and postmortem histopathological findings in a patient with normal-pressure glaucoma, including evidence of immunoglobulin deposition in the retina and apoptotic retinal cell death using terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) technique.

RESULTS

The histopathological changes of the optic nerve head and retina were more severe in the left eye of the patient with normal-pressure glaucoma than those seen in the right eye that were correlated with the clinical appearance of the optic discs. Both optic nerve heads exhibited remarkable cupping characterized by disarrangement, compression, and backward bowing of lamina cribrosa. The number of axons passing through the nerve head were decreased, and there were compact bundles of extracellular matrix. However, in the normal eyes, the lamina cribrosa displayed a regular horizontal arrangement. In both eyes with normal-pressure glaucoma, there were empty spaces in the optic nerve suggestive of

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MATERIALS AND METHODS

CLINICAL FINDINGS

A 74-year-old, white woman received a diagnosis of normal-pressure glaucoma and was followed up for 5 years. The initial diagnosis was based on the presence of open iridocorneal angles, no evidence of IOP greater than 20 mm Hg without antiglaucoma treatment, progressive glucovasomatus changes in visual fields and optic disc cupping, and absence of alternative causes of optic neuropathy. Alternative causes of optic neuropathy (ie, meningeval disease, infection, inflammation, ischemia, demyelinization, or compressive lesions) were excluded using neuro-ophthalmological examination with magnetic resonance imaging. She had coronary artery disease and family history of glaucoma. During the last 2 years of follow-up, she received topical β-blocker treatment in an effort to lower IOP from the middle to low teens and to retard progressive glucovasomatus optic neuropathy.

During the initial diurnal IOP measurements (3 times between 6 AM and 3 PM and 3 times between 5 PM and 6 AM) and during regular visits every 3 to 6 months at which measurements were obtained using applanation tonometry, IOP readings never exceeded 20 mm Hg. A visual field analyzer, 30-2 program (Humphrey Instruments, San Leandro, Calif) was used for visual field examinations. Initial visual field defects were characterized by bilateral nasal steps and dense paracentral scotoma in the left eye close to fixation. These defects progressed compared with baseline values based on the glaucoma change probability analysis. In Figure 1, the last stereoscopic optic disc photographs of the patient taken 1 year before the date of death and first and last results of visual field testing (5-year interval) are shown. The patient had bilateral, large optic disc cups (larger in left than right eye) that further enlarged during follow-up. During regular optic disc examinations, recurrent optic disc hemorrhages were observed in both eyes. The patient had also bilateral advanced parapapillary chorioretinal atrophy consisting of the α and β zones.11 Results of indocyanin green angiography and early stages of fluorescein angiography showed nonperfusion areas of the choriocapillaris in the parapapillary region. Fungus fluorescein angiography demonstrated a window defect corresponding to zone α of parapapillary atrophy, in which there are pigmentary and structural changes of the retinal pigment epithelium. One of the nonperfusion areas that was located adjacent to the inferotemporal optic disc border corresponded to the more advanced zone of parapapillary atrophy (zone β). In the later stages of the fluorescein angiography, fluorescein diffusion was seen in the previously nonperfused area of the more advanced zone of parapapillary atrophy extending to optic disc (Figure 2).

Laboratory studies revealed the presence of abnormal humoral autoimmunity, as demonstrated by an IgA paraproteinemia in addition to anticrocitolipin antibodies. To study the possible presence of antiretinal antibodies in serum, we performed immunoblotting using retinal substrates as previously described.12 Results of Western blot analysis and enzyme-linked immunosorbent assay showed the presence and high titers of circulating antibodies against several retinal proteins, including rhodopsin and heat shock proteins (hsp). Antibodies to retinal hsp included those directed to human and bacterial hsp60, hsp27, and α-crystallin (Figure 3).

METHODS

Postmortem eyes of our patient with normal-pressure glaucoma and, for comparisons, of a 72-year-old, white female donor with no history of ocular or neurological disease were obtained. All eyes were enucleated within 4 hours of death and processed within 12 hours. All eyes were fixed in 10% formalin, processed, and embedded in paraffin. Eyes were sectioned in the coronal plane from the distal end of the optic nerve to the equator. Serial sections, 4 µm thick, were prepared. Some of the sections were stained with hematoxylin-eosin and examined under a light microscope. Some of the sections were used to identify the apoptotic cells or for immunohistochemical analysis.

Identification of the apoptotic cells was performed using TUNEL technique, an in situ end-labeling technique for apoptotic cells that couples 2 major approaches: morphological examination and DNA fragmentation.13 It is a sensitive and specific technique that allows precise and rapid identification and quantification of the cell population involved in apoptotic death. Using an in situ cell death detection kit (Boehringer Mannheim, Mannheim, Germany), deparaffinized sections were incubated with a mixture of fluorescein-labeled nucleotides and terminal deoxynucleotidyl transferase (TdT) from calf thymus for 1 hour. The TdT catalyzes the polymerization of labeled nucleotides to free 3'-hydroxyl terminals of DNA fragments. A fluorescence microscope (Olympus, Tokyo, Japan) was used to visualize the apoptotic cells at the end of this period. Sections incubated with fluorescein-labeled nucleotide mixture without TdT served as a negative control. Sections previously treated with DNase I (1 mg/mL) to induce breaks in the DNA strands served as a positive control.

The sections were also examined using Ablin blue to identify mucopolysaccharides, Masson trichrome to outline the areas of gliosis, Luxol fast blue to delineate the myelin sheaths of the optic nerve, phosphotungstic acid–hematoxylin to identify fibrin deposits in blood vessels, and Congo red to examine perivascular amyloid deposits. We also performed immunohistochemical analysis to investigate the immunoglobulin deposition in the retina and optic nerve using antibodies against human IgG and IgA. For immunostaining, deparaffinized sections were incubated with proteinase K (20 µg/mL) for 20 minutes at room temperature. The samples were then treated with 3% bovine serum albumin at 37°C for 30 minutes to block nonspecific binding sites. After several washes, they were incubated at 37°C for 1 hour with fluorescein-conjugated monoclonal antibodies against human IgG or IgA (dilution, 1:16) (Sigma Chemical Company, St Louis, Mo). At the end of the incubation time, the sections were washed several times and examined using the fluorescence microscope. Age-matched healthy control eyes and antibodies against mouse immunoglobulins were used as negative controls.

Examination of serial hematoxylin-eosin–stained sections of the retina from the patient with normal-pressure glaucoma revealed a significant loss of retinal ganglion cells and their axons compared with that of the

Schnabel cavernous degeneration that stained with Alcian blue (Figure 4). In the parapapillary area, the retinal pigment epithelium, choriocapillaris, and photoreceptors were atrophic (Figure 2).
control eyes. In addition, the thickness of the inner nuclear layer (3-4 cell layers) appeared to be diminished compared with that of control eyes (8-9 cell layers) (Figure 5 A). Examination of the retinal sections revealed occasional retinal cells with nuclear or cytoplasmic condensation, pyknotic nuclei, or apoptotic bodies. The TUNEL technique showed brightly fluorescein-stained nuclei representing fragmented DNA and nuclear chromatin condensation (Figure 5, B and C). The TUNEL-positive cells were found mostly in the ganglion cell layer of the retina; however, a few cells were found in the inner and outer nuclear layers. The TUNEL-positive ganglion cells were sparsely distributed, corresponding to 0.1% of the total number of ganglion cells in each section. The TUNEL-positive cells were virtually absent in the age-matched control eyes.

The inner retina and optic nerve head demonstrated a decrease in the number and volume of capillaries compared with the control eyes, especially in some areas that exhibited significant loss of ganglion cells and their axons. However, patent capillaries were still present, and normal red blood cells were seen in those vessels. On the right posterior laminar area of the optic nerve, scattered structures resembling the vascular lumen without endothelial cells (Figure 6) and adjacent areas of axonal swelling and microglial infiltrations.
tion were noted. However, no amyloid or fibrin deposition was demonstrated along vessel walls, and the general loss of capillaries appeared proportionate to the loss of neural tissue in the retina and optic nerve head. The filling defects seen during angiography appeared to correlate with these findings.

Immunostaining was observed with anti–human IgG and IgA antibodies in the retina and optic nerve head of the eyes with normal-pressure glaucoma, which demonstrates the presence of serum immunoglobulins in these tissues. Immunostaining was noticeable in ganglion cells and in the inner and outer nuclear layers of the retina. Control antibody did not stain these tissues, and the control eyes did not exhibit immunostaining with any of the antibodies used (Figure 7).

**COMMENT**

We observed a disarrangement of the lamina cribrosa in our patient with normal IOP and glaucoma, similar to that described in patients with primary open-angle glaucoma. Similarly to our findings, Iwata has reported histopathological changes of the optic nerve head in normal-pressure glaucoma that were characterized by the disarrangement and backward bowing of the lamina cribrosa and loss of nerve fibers without evi-
ence of vascular abnormality. Furthermore, histo-
pathological optic nerve head changes correlated with
the clinical appearance of the optic nerve head that is
comparable in glaucoma with high and with normal
IOP. It seems, then, that there are complex mecha-
nisms related to individual anatomical, vascular, or
other differences in the susceptibility to damage that
result in similar changes of optic nerve head in glau-
coma with high and normal IOP.

Several previous studies suggest the importance of
the structural support of the lamina cribrosa and its role
in optic nerve fiber damage resulting from distortion of
the cribiform plates by elevated IOP. In postmortem glau-
comatous eyes with high IOP and in experimental glau-
coma models, there are dramatic changes in the lamina
cribrosa, eg, disarrangement and remodeling. However,
it is not known whether these changes play a causal
role in neural damage or whether they occur as a result
of the rearrangement of optic nerve head tissues second-
ary to elevated IOP, glaucomatous neural tissue loss,
and/or astroglial activation to rescue neural cells. The simi-
lar appearance of the lamina cribrosa in our patient to
that in eyes with high IOP further suggests that, in many
patients, the lamina cribrosa cannot sustain itself against
elevated or normal IOP because of progressive weak-
ness of the laminar beams, which may have a role in the
increased vulnerability of remaining axons to mechanical
forces.

Pathological studies of glaucomatous eyes from
humans and experimental glaucoma models have demon-
strated Schnabel cavernous degeneration in the optic
nerve, which is characterized by the disappearance of
axons, the accumulation of mucopolysaccharide within
cavernous spaces, and the absence of macrophagic or
glial proliferative reaction similar to that observed in
our patient with normal-pressure glaucoma. These fea-
tures of cavernous degeneration are consistent with the
apoptotic cell death and optic disc cupping that may
develop secondary to the collapse of these cavernous
spaces.

In experimental glaucoma models with elevated IOP,
retinal ganglion cell death occurs mostly by apopto-

Figure 3. Results of Western blot analysis of a patient with normal-pressure
glaucoma. Each lane contains patient serum (dilution, 1:1000) against bovine
retinal supernatant (BRS), bovine retinal membrane (BRM) (15 µg/lane),
purified αA- and αB-crystallin, heat shock protein (hsp)27, bacterial (B) and
human (H) hsp60, and rhodopsin (3 µg/lane), as labeled. Secondary
antibody (goat anti-human IgG) dilution is 1:2000.

Figure 4. Optic nerve head in normal-pressure glaucoma. A, Optic disc
cupping, posterior bowing of the lamina cribrosa (arrowheads), and
Schnabel cavernous degeneration in the prelaminar (small arrows) and
postlaminar (large arrows) areas of the right optic nerve head (Masson
trichrome; original magnification ×25). B, Extensive Schnabel cavernous
degeneration in the postlaminar area of the left optic nerve (S). Posterior
bowing of the lamina cribrosa (arrowheads) is seen (hematoxylin-eosin;
original magnification ×10). C, Cavernous areas in the left optic nerve
(Alcian blue; original magnification ×25).
sis. Postmortem studies of human eyes with primary open-angle glaucoma also suggest apoptosis as a mechanism of retinal ganglion cell death. Similarly, optic nerve transection may lead to apoptosis in retinal ganglion cells. Apoptotic retinal cell death may also be initiated with ischemia, as seen in anterior ischemic optic neuropathy or diabetes. The fact that apoptotic retinal ganglion cell death, cavernous degeneration of optic nerve, and excavation of the optic disc seen in glaucomatous optic neuropathy all may occur in anterior ischemic optic neuropathy suggests a shared common mechanism. However, there was no significant sign of vascular abnormality in our patient with normal-pressure glaucoma, except some scattered structures in one of the optic nerve heads resembling vascular lumen without endothelial cells.

We hypothesize that autoantibodies directed toward retinal antigens, as seen in our patient, may play a previously unrecognized role in facilitating apoptotic cell death in some patients with glaucomatous optic neuropathy, particularly those with normal IOP and evidence of serum abnormalities of humoral immunity. The serum from many patients with normal-pressure glaucoma contains higher titers of specific antibodies against several retinal proteins, including rhodopsin and hsp. Heat shock proteins are a family of cellular chaperone proteins of varying molecular weights that are considered neuroprotective, since their expression is induced in neurons to ameliorate damage in response to a variety of stress conditions, eg, ischemia and excitotoxicity. Furthermore, they are highly antigenic, and the immune response to these proteins may have protective and pathogenic potential. The immune responses to hsp are implicated in the development of a number of human autoimmune diseases, including several types of inflammatory arthritis, type 1 diabetes mellitus, and multiple sclerosis.

Mammalian retinal ganglion cells in culture express hsp72 in minutes to hours after exposure to hypothermic or hypoxic conditions, and this treatment renders the cells somewhat resistant to subsequent anoxia or glutamate treatment. Compelling evidence that retinal hsp are among the significant autoantigens in patients with glaucoma has recently been discovered. We propose that increased titers of circulating antibodies against retinal hsp, such as we have identified in our patient (Figure 3), may predispose toward retinal neuronal loss or damage of the vasculature of the retina or optic nerve due to antibody inactivation or attenuation of endogenously released retinal hsp. In this manner, high titers of autoantibodies to retinal hsp may contribute to glaucomatous optic neuropathy in these patients.

A previous report described an increased prevalence of monoclonal gammopathies in patients with normal-pressure glaucoma (12% in our current cohort) and hypothesized that the increased prevalence of paraproteinemias in patients with normal-pressure glaucoma suggests that autoimmunity may have a role in the glaucomatous disease process in these patients. Although the presence of a monoclonal protein in the

Figure 5. Evidence of apoptosis in normal-pressure glaucoma. A, Loss of ganglion cells and their axons in the midperipheral retina of the patient with normal-pressure glaucoma. Arrowhead shows a ganglion cell with pyknotic nucleus and intact cytoplasm; arrows show a ganglion cell with condensed nucleus and shrunken cytoplasm (hematoxylin-eosin; original magnification ×100). B, Cells positively labeled using terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) technique (arrowheads) in the ganglion cells and inner nuclear layers of the retina sectioned from the eyes with normal-pressure glaucoma (original magnification ×40). C, Cells positively labeled using TUNEL technique (arrow) in the ganglion cells layer of the retina (original magnification ×100).
serum of a patient with a peripheral neuropathy may be associated with systemic lymphoproliferative disorders (eg, multiple myeloma or lymphoma), the neuropathy is classified as a monoclonal gammopathy of undetermined significance if these disorders are excluded by appropriate studies, including bone marrow aspiration. Our patient’s monoclonal gammopathy has been characterized as monoclonal gammopathy of undetermined significance.

Our finding of immunoglobulin deposition in the retina is the first such report in a patient with normal-pressure glaucoma and paraproteinemia. The potential pathogenic significance of paraproteinemias for glaucomatous optic neuropathy lies in the observation that a well-recognized spectrum of insidious, slowly progressive peripheral sensory and motor neuropathies has been demonstrated to be associated with benign monoclonal IgG, IgM, and IgA paraproteinemias. These paraproteins are considered to be likely causative agents of these peripheral neuropathies in which the neural antigenic targets of these proteins have been identified. In addition, there appears to be great similarity of the clinical course of patients with peripheral neuropathy and monoclonal gammopathy to that of patients with glaucoma. For example, the patients with peripheral neuropathy have reproducible features, with a symmetric sensory motor polyradiculopathy or neuropathy that is slowly progressive during months or years, and with a median age of 55 to 60 years.

Approximately 20 years ago, a patient with peripheral neuropathy and IgM gammopathy was described, and IgM and IgM-producing lymphocytes were shown to have infiltrated the peripheral nerves. By 1982, 58 cases of monoclonal gammopathy were described in association with peripheral neuropathy. The demonstration of immunoglobulin deposition in peripheral nerves, combined with the recognition of a higher than usual prevalence of patients with paraproteinemia associated with peripheral neuropathy, was seminal in the recognition of the potential role of an autoantibody in the disease process. Unlike patients with peripheral neuropathies, patients with glaucoma cannot routinely be subjected to optic nerve biopsy to investigate the pathogenesis of their disease. We are therefore fortunate for the rare opportunity to study the ocular tissues in our patient with normal-pressure glaucoma and paraproteinemia promptly after her death. In our patient, immunoglobulin deposition in the ganglion cell layer and in the inner and outer nuclear layers may signify that proteins in these cell layers act as putative autoantigens in which an aberrant systemic humoral immune response has occurred.

Retinal immune deposits have been observed previously in cancer-associated retinopathy syndrome and have been suggested as a causative factor for retinal degeneration in these patients in whom there is no clinical evidence of a breakdown of the blood-retina barrier. However, we speculate that parapapillary chorioretinal atrophy commonly seen in patients with glaucoma, in which the outer blood-retina barrier is impaired, as supported by the clinical and pathological findings of our patient, may allow the circulating antibodies access to retinal antigens in these patients.

There is considerable evidence that autoantibodies can cause neuronal apoptotic cell death. It has been shown in many autoimmune diseases of the central nervous system that the specific interaction of an antibody with a target antigen or with voltage-gated channels (ie, Na+, Ca++, and K+ channels) present on neural cells can result in selective neurological degeneration or dysfunction in these diseases. In the eye, human serum antibodies specific to the retinal protein recoverin may enter retinal cells and cause apoptotic cell death.

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Figure 7. Immunostaining of the retina from the patient with normal-pressure glaucoma with fluorescein-conjugated monoclonal antibodies to human immunoglobulins. Immunostaining with antibodies to human IgA (A) or IgG (C) is visible in the ganglion cells (g) and outer (on) and inner nuclear (in) layers of the retina sectioned from the patient. Fluorescein staining is not seen in the age-matched control eyes with antibodies to human IgA (B) or IgG (D) except normal autofluorescence.