A Histopathologic and Morphometric Differentiation of Nerves in Optic Nerve Hypoplasia and Leber Hereditary Optic Neuropathy

Hossein G. Saadati, MD; Hugo Y. Hsu, AB; Keith B. Heller, BS; Alfredo A. Sadun, MD, PhD

Objectives: To characterize and quantitate optic nerve histopathologic and morphometric differences between optic nerve hypoplasia (ONH) as an early and congenital form of intrinsic axonal loss and Leber hereditary optic neuropathy (LHON) as a late and acquired form of intrinsic axonal loss.

Materials and Methods: Optic nerves from 3 sources were examined: a 42-year-old healthy woman (control), a 53-year-old woman with ONH diagnosed postmortem, and a 74-year-old woman with LHON. The optic nerves were processed, embedded, and stained with a 1% solution of paraphenylene diamine. Histopathologic and morphometric analyses were performed via light microscopy and a semiautomatic computer image analysis system.

Results: The ONH showed severe axonal depletion without degenerated profiles in an inferonasal sector, with only a small superotemporal sector having a near normal appearance. The LHON revealed general axonal depletion centrally, fibrocytic scarring, scattered “degeneration dust,” and evidence of minimal inflammation, with residual axons limited to superior and temporal peripheral clusters. Morphometric analysis revealed total fiber populations of 98,000 in the ONH optic nerve and 48,000 in the LHON optic nerve, representing 90% and 95% reductions, respectively, compared with the control optic nerve (1.2 million fibers).

Conclusions: Optic nerve hypoplasia and LHON present 2 distinguishable and distinctive patterns of nerve fiber distribution and axonal dropout. The lack of degenerated axons in ONH indicates that any axonal death probably occurred through apoptosis during development. In LHON, degenerated axons and minimal grade of inflammation were obvious, implicating a more “active” pathologic process. This study describes distinctions between these 2 optic neuropathies.


A DEARTH of literature exists regarding detailed histopathologic or morphometric descriptions of optic nerve hypoplasia (ONH) and Leber hereditary optic neuropathy (LHON), much less a comparison of these early and late forms of intrinsic axonal loss.

Optic nerve hypoplasia was first recognized in 1915; however, detailed clinical descriptions were not available until the 1960s. Initially, ONH was thought to be a rare anomaly; more recently, it has been regarded as a fairly common cause of blindness in children. Optic nerve hypoplasia is a nonprogressive congenital defect that can occur unilaterally or bilaterally, either in isolation or in association with other central nervous system malformations and endocrine malfunctions. The incidence of ONH is equal in males and females. It can manifest in complete, incomplete, or segmental forms. Optic nerve hypoplasia is usually idiopathic, although the use of several toxins, medications, and alcohol during pregnancy, as well as maternal diabetes mellitus, all have been associated with children born with ONH. The characteristic clinical features range from severely decreased vision usually discovered in infants to an incidental finding without visual loss with or without mild visual field abnormalities and reduced pupillary response to light in adults. Results of funduscopic examination reveal a small optic disc and often the so-called double ring sign.

Unilateral or asymmetrical cases of ONH may be misdiagnosed as simple primary strabismus and amblyopia. Bilateral cases may be erroneously diagnosed as being one of the hereditary congenital forms of optic atrophy but should be primarily differentiated from Leber congenital amaurosis and achromatopsia, which may have similar clinical features. Leber congenital amaurosis is not to be confused with the optic nerve hypoplasia found in children.
MATERIALS AND METHODS

In this study, 3 optic nerves were carefully examined. The first was obtained from a 42-year-old woman without a history of any neurologic or ophthalmic problems who had died of hepatic failure. The second optic nerve was obtained from a 53-year-old woman with ONH who had been blind since birth. Her medical records from 1964 to 1973 indicated that she was initially misdiagnosed at age 20 years with “Leber disease” (some of the physician’s notes incorrectly implied LHON rather than ONH). However, the same records revealed that her visual acuities had been fairly stable (20/100 OD and 20/200 OS) since early childhood. Her fundus examination results were described as bilateral optic nerve atrophy. However, results of a skull x-ray (performed in 1970) were negative, and there was no family history of any neuro-ophthalmologic disease. At age 53 years, the patient died of extensive third-degree thermal injuries, and the eyes, optic nerves, and brain were obtained and preserved in 20% formalin within 3 hours of death. Gross and histoanatomical examination of the brain and histopathologic examination of the eyes were performed.

The third optic nerve was obtained from a 74-year-old woman known to have the 11778 primary mutation of LHON. She experienced a painless loss of vision at age 38 years in 1 eye; then, within 3 weeks, her vision declined to light perception in both eyes and remained at this level until she died of severe chronic obstructive pulmonary disease.

All optic nerves were fixed, osmicated, dehydrated in ethanol and propylene oxide, and embedded in epoxy resin (Epon). The left optic nerves were sectioned on an ultramicrotome at a thickness of 1.0 to 1.5 mm and stained with a 1% solution of paraphenylenediamine, which stains for myelin and shows axonal degeneration.15,16

Stained cross-sections of optic nerves were examined with light microscopy for histopathologic comparisons, and morphometric analysis of fibers (axon with myelin sheath) was performed via a semiautomatic computer image analysis system with video acquisition and digitalization as previously described.13,19 The total fiber population of the optic nerves was calculated from multiple sampling in each of 16 sectors and in the total cross-sectional area of the optic nerve as described previously.13,19 Despite the fact that the number of optic nerves was only 3, there remained a statistically significant difference because of the large population of fibers counted in each nerve.

RESULTS

Light microscopic and morphometric analysis of the control optic nerve revealed a diameter of 2.1 mm (Figure 1), a normal nerve fiber size spectrum, and a total count of 1.2 million fibers (Table).

Results of gross examination of the brain of the patient with ONH showed hypoplastic changes in the corpus callosum limited to the posterior portion (splenium). Results of gross examination of the eyes revealed (1) a peripheral cataract, (2) some atrophic changes in the ciliary bodies, and (3) flat and pale optic nerves with sclerotic vessels at the optic nerve head.

Results of light microscopy revealed severe axonal depletion largely limited to a large, inferonasal sector (Figure 2, left) that contained an anomalous small artery distinct from the central artery (Figure 2, right). Only a small superotemporal sector contained axons compacted to a near normal appearance. The remaining sectors consisted of sparse scattering of axons with extensive proliferation of astrocytes, with no apparent

Figure 1. Cross-section of control retrobulbar optic nerve (1% paraphenylenediamine solution, × 80).

Table

<table>
<thead>
<tr>
<th>Fiber Diameter</th>
<th>Number of Fibers</th>
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<td>1.0 to 1.5 mm</td>
<td>1.2 million</td>
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degenerated profiles or macrophages detected (Figure 2, right). Results of morphometric analysis revealed a reduced overall diameter of the ONH compared with the control (1.5 mm vs 2.1 mm) and a total fiber population of only 98,000, representing a 90% reduction compared with the control optic nerve (P < .001) (Table).

In contrast, results of light microscopic examination of the LHON revealed a diffuse and general depletion of fibers involving all of the central portion, with residual axons limited to peripheral clusters superiorly and temporally (Figure 3, left). The central portion of the optic nerve, significantly depleted of axons, consisted of fibrocytic scarring and scattered degeneration dust associated with macrophages (Figure 3, right). In contrast to the ONH case, the LHON displayed profiles of degenerated axons, fewer astrocytes, and an increased number of fibroblasts.

Results of morphometric analysis showed a reduced overall diameter of the LHON compared with the control (1.6 vs 2.1 mm) and a total fiber count of only 48,000, representing a 95% reduction compared with the control optic nerve (P < .001) (Table).

### Histological and Morphometric Comparison of Control, Optic Nerve Hypoplasia (ONH), and Leber Hereditary Optic Neuropathy (LHON) Optic Nerves

<table>
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<tr>
<th>Optic Nerve</th>
<th>Control</th>
<th>ONH</th>
<th>LHON</th>
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<tr>
<td>Counted fiber population*</td>
<td>1,200,000</td>
<td>98,000</td>
<td>48,000</td>
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<tr>
<td>Total cross-sectional area, mm²</td>
<td>3.5</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Pattern of axonal distribution</td>
<td>Normal</td>
<td>Mostly superotemporal and a thin sector inferiority</td>
<td>Peripheral clusters superiorly and temporally</td>
</tr>
<tr>
<td>Degeneration pattern</td>
<td>No degeneration</td>
<td>No degeneration</td>
<td>Scattered “degeneration dust” and some 1% paraphenylene diamine solution–verified degeneration</td>
</tr>
<tr>
<td>P (fiber population vs normal optic nerve)</td>
<td>†</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

* Axon + myelin.
† Ellipses indicate no data to be entered.

**Figure 2.** Left, Cross-section of retrobulbar optic nerve with optic nerve hypoplasia showing axonal depletion in a large inferonasal sector (arrows) (1% paraphenylene diamine solution, original magnification × 80). Right, High-power picture from an axon-depleted area showing no apparent axonal degeneration or macrophages (1% paraphenylene diamine solution, original magnification × 800). This area also contains an anomalous small artery with elastin distinct from the central artery (arrow).
Results of morphometric comparisons of the nerve fiber counts and nerve fiber diameter spectra in ONH, LHON, and control optic nerves revealed a significant shift of fiber diameters to smaller sizes in LHON optic nerves (histogram). The line diagram (inset) in Figure 4 shows the relationship between the modes of each nerve fiber spectrum, with a shift to the left in LHON.

COMMENT

Earlier studies of ONH and LHON optic nerves provided only limited histopathologic descriptions. Reports of ONH eyes have described subnormal optic nerve diameters and depletions of retinal ganglion cells and axons in the nerve fiber layer and optic nerve.6,20 Regarding LHON, previous reports revealed a reduction of the optic nerve diameter, a central depletion of axons with residual peripheral clusters of axons, and fibrillary gliosis10 without detectable inflammatory profiles.21 We previously reported a reduction of the optic nerve diameter, a central axonal loss, and minimal inflammatory changes in an optic nerve from a patient with the 11778 primary mutation of LHON.15 Kerrison et al22 also recently examined an optic nerve from a patient with LHON. They showed that optic nerve atrophy did not appear more prominent centrally than peripherally, and they did not detect any inflammatory changes.22 They described calcium inclusion bodies as possible evidence of mitochondrial involution. However, we failed to corroborate this.23

The possible underlying mechanisms previously suggested regarding ONH have, in the absence of ultrastructure, been partially speculative. Most early investigators thought that ONH resulted from a failure of the retinal ganglion cell layer to differentiate between the 13- and 17-mm stage of embryonic development.1,9,20,24 In 1957, Mann25 suggested that when retinal ganglion cell axons fail to form central connections, these axons then degenerate, which, in extreme cases, results in ONH. In 1978, Moiser et al6 examined the eyes of a child with hydranencephaly and ONH and found in the retinas normal amacrine and horizontal cells; they argued that failure of retinal ganglion cell differentiation as a cause of ONH was unlikely because all 3 cell types arise from the same stem cells. Alternatively, in 1978 Frisen and Holmegaard4 suggested that ONH is a consequence of degeneration of ganglion cells and their axons caused by an insult to the developing visual pathway. This would not be contradictory to the general concepts of Mann25 regarding the process of retinofugal specificity acquisition. However, despite these theories, the pathogenesis of ONH remains incompletely understood.

Leber hereditary optic neuropathy was initially believed to be a rare, X-linked genetic disorder. However, recent studies have confirmed that the pathogenesis of LHON is caused by point mutations in the mitochon-
drial DNA, producing mitochondrial dysfunction. Harding et al suggested that optic nerve damage in LHON could be immunologically mediated and that mitochondrial genes might contribute to susceptibility of multiple sclerosis. Results of our histopathologic examination did not reveal any signs of perivasculitis, and the detectable inflammatory changes were minimal. Hence, we could not corroborate the suggestion of Harding et al of an immunologic component for optic nerve damage in LHON.

In the present study, results of light microscopic examination of the optic nerve from our patient with ONH revealed sectoral axonal loss without any detectable degenerated profiles, consistent with the more limited descriptions in the literature. The present study of an optic nerve with LHON showed central axonal loss with detectable fibrocytic scarring and scattered degeneration dust with minimal inflammatory profile, contrary to previous findings.

The data from morphometric analysis and comparison of control, ONH, and LHON are summarized in the Table. Morphometric comparison of nerve fiber diameters from healthy, ONH, and LHON reveals a significant shifting of nerve fiber diameters to the smaller size in LHON, notwithstanding the fact that the nerve fiber diameter is expected to be of greater caliber in the peripheral portions of the optic nerve. On the other hand, the nerve fiber diameter spectrum in ONH was similar to that of the control optic nerve. The shifting of the nerve fiber diameter in LHON is intriguing. We had anticipated a shift in the opposite direction insofar as clinically, LHON involves a selective loss of the smaller papillomacular fiber. One possible explanation is that surviving axons diminish in caliber.

The corpus callosum (splenium) anomaly in our patient with ONH suggests a more generalized neurodevelopmental impairment, consistent with the study by Novakovic et al. Our patient with ONH was originally misdiagnosed as having Leber disease. Such confusion probably stems from the difficulty in differentiating ONH from Leber congenital amaurosis (the name Leber has been applied to 2 forms of optic neuropathy).

Yet, as our study results show, ONH and LHON are distinguishable not only clinically but also histopathologically. Each has a clearly distinctive pattern of nerve fiber distribution and axonal dropout. The histopathologic findings in ONH did not reflect evidence of axonal degeneration. These findings and the previously accumulated experimental and clinical data suggest that ONH may be the result of excessive “apoptosis” of the retinal ganglion cells during the development of visual pathway, which fits in well with Mann’s concept of the overproduction of retinal ganglion cells and their axons, followed by the dying back (or apoptosis) of those that fail to establish the correct terminal connections. Alternatively, as Frisen and Holmegaard suggested, it is possible that ONH is caused by an insult directly to the developing anterior visual pathway, which leaves only a small number of nerve fibers intact.

Conversely, in LHON, mutational-induced mitochondrial dysfunction directly leads to axonal degeneration and an associated minimal inflammation, presumably implicating a more degenerative process with a mild reactive immunologic component in LHON.

The association between ONH and LHON probably reflects 2 distinct pathogenetic mechanisms: the result of apoptosis and the result of specific degenerative processes, respectively.

The present study had 2 important limitations. Pathologic condition described at the end of a long life may not reflect the processes that occurred 6 decades earlier. Only 1 optic nerve was available as a sample of each of

![Figure 4. Histogram of nerve fiber counts vs nerve fiber diameter (with inset of line diagram) from healthy (control), optic nerve hypoplasia (ONH), and Leber hereditary optic neuropathy (LHON) optic nerves. The line diagram shows the relationship of modes of each nerve fiber spectrum. Note the shifting of the mode to the left, especially in Leber hereditary optic neuropathy.](http://archophth.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/6613/ 06/03/2017)
these optic neuropathies. Further histopathologic and morphometric comparison of the retina, lateral geniculate nucleus, and other primary visual nuclei from the patients with ONH and LHON might, compared with healthy controls, provide more information regarding the pathogenesis of these clinical entities.

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Reprints: Alfredo A. Sadun, MD, PhD, Doheny Eye Institute, University of Southern California School of Medicine, 1450 San Pablo St, Los Angeles, CA 90033.

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REFERENCES


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Announcement

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