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ORIGINAL ARTICLE *free on line*

Retinal nerve fiber layer thickness variability in Leber hereditary optic neuropathy carriers

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Purpose. *Recent investigations suggested that unaffected carriers of Leber hereditary optic neuropathy (LHON) may show subclinical visual alterations. Structural changes have also been detected by optical coherence tomography (OCT), which revealed a temporal thickening of the retinal nerve fiber layer (RNFL). These changes may reflect compensatory effects such as mitochondria accumulation within the RNFL axons. This study aimed to investigate whether the RNFL of LHON carriers shows greater than expected thickness variations, which may reflect transient subclinical changes, over the course of years.*

Methods. *Using Stratus OCT, the RNFL thickness was measured yearly from 2005 to 2008 in 24 Brazilian LHON carriers, all with homoplasmic 11778/ND4 mtDNA mutation. An Italian sample of 20 healthy subjects served as a control. Data were compared also to a previously published sample (n=59) of glaucomatous eyes.*

Results. *The LHON carriers showed test-retest standard deviations that were larger than normal controls in the temporal (p=0.004), superior (p<0.0001), and inferior quadrants (p=0.019). Compared to the glaucoma cases, no statistical differences were observed.*

Conclusions. *The RNFL thickness in LHON carriers, when measured at different time points, has higher variability than in normal subjects. Transitory RNFL swelling may be caused either by compensatory mechanisms (increased mitochondrial biogenesis) or by axonal stasis preceding decompensation of retinal ganglion cells. In both situations, these changes may represent the origin of the visual alterations previously detected in LHON carriers. Alternatively, increased variability of RNFL thickness may be influenced by the LHON microangiopathy, as retinal blood vessels contribute to the OCT RNFL thickness measurements.*

Key Words. *Leber hereditary optic neuropathy, Retinal nerve fiber layer thickness*

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INTRODUCTION

Leber hereditary optic neuropathy (LHON) is a maternally inherited genetic disorder caused by mitochondrial DNA (mtDNA) pathogenic mutations that affect complex I, the first site of the mitochondrial respiratory chain. Visual loss in LHON is related to the selective degeneration of retinal ganglion cells and their axons, which undergo an acute thickening at the onset of the disease and a progressive thinning as atrophy develops in the following months. Eventually, LHON results in bilateral large cecocentral scotomas (1, 2).

The mtDNA mutation is necessary, but not sufficient, to produce the disease; other, still poorly defined, factors, such as environmental or genetic, are believed to contribute (1). A recently identified anatomic trait, optic nerve head size, has also been implicated in predisposing to disease or affecting its severity (3). Thus, penetrance in LHON is not complete and the majority of maternally related individuals along a given maternal lineage and carrying a homoplasmic mtDNA pathogenic mutation (LHON carrier) do not develop the optic neuropathy (1). Although these carriers are usually defined "unaffected," recent investigations have suggested that subclinical or occult alterations may occur, such as paracentral or arcuate scotomas on visual field testing, reduction in spatial contrast sensitivity, dyschromatopsia, depressed central response, abnormal interocular asymmetries in multifocal visual evoked response and multifocal electroretinogram, and prolonged latencies with larger temporal dispersion in pattern-reversal visual evoked potentials (Salomao SR, et al. IOVS 2003;44:ARVO E-Abstract 936) (4-6). Structural abnormalities of the optic nerve, possibly related to these subclinical changes, have not been fully characterized. Using optical coherence tomography (OCT), our group previously detected a temporal thickening of the retinal nerve fiber layer (RNFL) in a large Italian sample of unaffected LHON carriers (7). These changes may reflect compensatory effects such as the accumulation of mitochondria within the axons that constitute the RNFL. Investigations of whether the RNFL of LHON carriers is subject to thickness variations over the course of years and in the absence of disease onset may reflect transient subclinical changes. Thus, RNFL longitudinal changes have the potential to predict the disease onset.

The International Field Investigation in Colatina, Brazil, an ongoing 10-year follow-up study of a very large LHON pedigree that carries the 11778/ND4 mutation, gave us the opportunity to measure the RNFL thickness in a population of Brazilian LHON carriers all with the same mtDNA. Measurements were taken yearly from 2005 to 2008. The present study aimed to assess whether the RNFL of these subjects showed thickness variability greater than that observed in healthy subjects using the same measurement technology.

METHODS

Subjects

Among the 65 Brazilian LHON carriers who had been examined through the years 2005 and 2008, we analyzed only those who had undergone OCT imaging of the optic nerve in all 4 years (n=24; mean age: 27.5±19.8 years; range 6-65 years). All individuals belonged to the maternal lineage of the SOA-BR family and invariably carried the homoplasmic 11778/ND4 mtDNA mutation on a haplogroup J background (8). The control group included 20 age-matched healthy volunteers (mean age 30.9±4.5 years; range 25-50 years) belonging to the staff of the Neurology Department of the University of Bologna.

All subjects had a comprehensive ophthalmic examination, including best-corrected visual acuity measurement, slit-lamp biomicroscopy, intraocular pressure measurement, indirect ophthalmoscopy, and optic nerve head photography. We excluded cases with presence in one or both eyes of any retinal and/or optic nerve disease, an intraocular pressure of ≥21 mm Hg, a history of amblyopia, refractive error of ≥5 D of sphere or ≥2.5 D of cylinder, a history of any disease, surgery, or trauma in the eye being tested, a history of systemic medication use (e.g., Plaquenil) that might affect the visual field, a history of a cerebrovascular event or diabetic retinopathy, a history of glaucoma in the family, and/or systemic diseases with possible ocular involvement, such as diabetes mellitus. In the control group, if both eyes were normal, one eye was randomly selected.

A third group of glaucomatous eyes (n=59, mean age = 68±13 years; range 29-88 years), previously analyzed by Budenz et al (9), was analyzed to evaluate the RNFL thickness variability in glaucoma.

All participants gave informed consent according to the Declaration of Helsinki and the study was approved by

the internal review board of the Department of Neurological Sciences at the University of Bologna and the institutional review board of the Federal University of Sao Paulo.

Instrumentation and procedures

All subjects underwent RNFL thickness measurement by OCT (Stratus OCT, software version 4.0.1; Carl Zeiss Meditec, Inc., Dublin, CA, USA), using the RNFL thickness 3.4 acquisition protocol and following the same procedure reported by Budenz et al (10). Technical details about the Stratus OCT imaging technology have been previously reported (11).

The standard RNFL Thickness 3.4 scan consists of 512 measurements taken in a circle around the optic disc, with a standardized diameter of 3.4 mm. For each eye, we obtained one scan at each time point during the study. We subsequently studied the average RNFL thickness, temporal quadrant thickness, superior quadrant thickness, nasal quadrant thickness, and inferior quadrant thickness, all automatically calculated by OCT using the existing software.

The examination was performed after mydriasis by 3 experienced operators (P.B. and C.R. for LHON carriers and M.C. for the control group). At the beginning of the examination the OCT lenses were adjusted for the patient's refractive error, which was within ±5 D of emmetropia in all subjects. Polarization was optimized to maximize the reflective signal and the best centration of the scan with respect to the optic disc was always utilized. Only good quality OCT data as judged by the appearance of the RNFL and the optic disc pictures were used for further analysis. Images with artifacts, missing parts, or showing seemingly distorted anatomy were excluded. Since the position of the circular scan with respect to the optic disc is crucial, we included only images where the optic nerve head (ONH) was well centered by the scan. Examinations with a signal strength lower than 6 were excluded.

Subjects belonging to the control group underwent the same examination on 3 different days during a period of 3 months.

Statistical analysis

All statistical analysis was performed with SPSS 17.0 (PASW, Chicago, IL, USA). Analysis of possible subclinical changes in LHON carriers proceeded along 3 lines:

1) The repeated measures data of the 24 participants with complete follow-up were included in a general linear model analysis accounting for the correlation within individuals to evaluate whether there was statistically significant progression of RNFL thinning over time. Budenz et al demonstrated that disease-free individuals experience a normal age-related thinning (12), which over the 3 follow-up years of this study should change average RNFL by –0.6 µm, on average. For this analysis the right and left eyes of participants were averaged.

2) Since no systematic change in RNFL thickness was found over time (see Results), we analyzed the 4 measurements between 2005 and 2008 as 4 intersession test-retest measurements, partitioning variance components into those due to differences between subjects and pooled within subject variability, that is, the test-retest variance. These were expressed as standard deviations and 95% confidence intervals were calculated for average RNFL thickness and thicknesses at each quadrant. These estimates were compared to those from the dataset of normal controls and a cohort of glaucoma patients measured in a similar fashion with the F test (13). Since these latter 2 datasets only included one eye per participant, one randomly selected eye of each LHON carrier was included in this analysis.

3) To evaluate whether greater than normal right eye minus left eye interocular differences were present, mean and standard deviations of interocular differences of RNFL measurements of the 50 participants with both eyes measured at baseline were compared against interocular differences found in disease-free individuals (13). Means were compared with the 2-sample t-test and squared standard deviations were compared with the F test.

RESULTS

Average rate of RNFL thinning in the cohort

Among the cohort, there was no indication of a progressive thinning beyond that expected by normal aging (slope ± SD –0.33±0.21 µm/y; 95% confidence interval –0.74, 0.09; p=0.13). Table I summarizes average RNFL thickness measurements by study year averaged over the 2 eyes of the 24 participants.

Changes in individual carriers beyond expected test-retest variability

The LHON carriers showed test-retest standard deviations that were larger than normal controls in the temporal, superior, and inferior quadrants; however, compared to the glaucoma cases, no statistical differences were observed. The nasal measurements varied the most and that was probably reflected in the average RNFL measurements (Tab. II, Fig. 1).

Baseline interocular differences

On average, right eyes were slightly thicker than left eyes (respectively, 108.8±10.9 and 107.2±11.4 μm) with a mean difference of 1.6±5.6 μm (p=0.050). Table III compares mean and standard deviation of interocular differences in LHON carriers versus the comparably measured cohort of normal controls. No statistically significant differences were found.

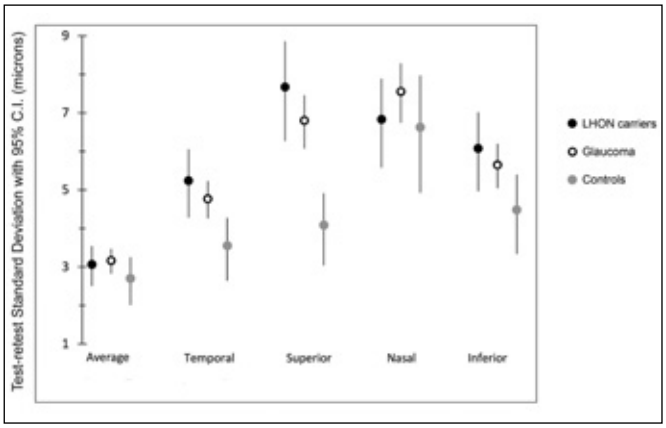

Fig. 1 - *Test-retest standard deviation of retinal nerve fiber layer thickness measurements (average and 4 quadrants) of Leber hereditary optic neuropathy (LHON) carriers, glaucoma patients, and controls.*

DISCUSSION

The peripapillary RNFL undergoes a characteristic pattern of changes in acute LHON. Concurrent with the subacute loss of vision, all quadrants show a significant thickening of the RNFL originally described by Nikoskelainen et al as optic nerve swelling (14). Recent studies with OCT revealed that the RNFL thickening involves asynchronously the 4 quadrants through a 3-month period, with the temporal and inferior sectors being affected first (2). In asymptomatic LHON carriers, these 2 same sectors were found to be significantly thicker, as measured by OCT (7). The nature of the RNFL thickening in both preclinical and acute patients remains unclear. The non-inflammatory pseudoedematous swelling of the RNFL is probably a combination of impaired axoplasmic transport and a compensatory increase of mitochondrial biogenesis, which first involves the unmyelinated portion of the retinal ganglion cell (RGC) axons. This process may start first in the most vulnerable axons and somehow propagate to the contiguous axonal bundles. The same hypothesis has been postulated to explain the RNFL thickening observed in LHON carriers (7).

In the present study, we show that the RNFL thickness in LHON carriers, when measured at different time points, has higher variability than in normal subjects. From a statistical point of view, this result is reinforced by the observation that no progressive thinning (beyond that expected by normal aging) was detected and that interocular differences were not significant (confirming previous findings by Budenz in disease-free subjects) (13). The higher variability may reflect a dynamic aspect of LHON, as transitory RNFL swelling may be caused either by compensatory mechanisms (with increased mitochondrial biogenesis) or by axonal stasis that may precede the catastrophic decompensa-

TABLE I - DESCRIPTIVE STATISTICS FOR AVERAGE RNFL THICKNESS (μm) BY YEAR OF STUDY IN THE LHON CARRIERS GROUP

Study year	Mean ± SD	Minimum	Maximum	Median
Baseline	106.7±11.9	86.0	133.9	107.0
Follow-up year 1	106.9±12.1	85.8	136.1	107.9
Follow-up year 2	106.7±11.9	86.6	131.3	109.5
Follow-up year 3	105.7±11.6	87.1	131.8	109.6

LHON = Leber hereditary optic neuropathy; RNFL = retinal nerve fiber layer

TABLE II - COMPARISON OF TEST-RETEST STANDARD DEVIATIONS OF LHON CARRIERS TO NORMAL CONTROLS AND GLAUCOMA PATIENTS

Location and group	Mean	Test-retest SD	Confidence limits		p value LHON carriers vs controls	p value LHON carriers vs glaucoma patients
			Lower	Upper		
Average						
Controls	100.72	2.70	2.01	3.25		
LHON carriers	106.86	3.07	2.50	3.54	0.194	0.615
Glaucoma patients	62.47	3.16	2.82	3.47		
Temp						
Controls	77.03	3.55	2.64	4.27		
LHON carriers	79.44	5.24	4.28	6.05	0.004	0.156
Glaucoma patients	52.03	4.77	4.26	5.23		
Superior						
Controls	122.12	4.08	3.04	4.91		
LHON carriers	128.76	7.67	6.26	8.86	<0.001	0.100
Glaucoma patients	73.10	6.80	6.07	7.46		
Nasal						
Controls	78.20	6.63	4.93	7.97		
LHON carriers	80.49	6.83	5.58	7.89	0.425	0.837
Glaucoma patients	53.80	7.55	6.74	8.28		
Inferior						
Controls	125.63	4.49	3.33	5.40		
LHON carriers	138.58	6.08	4.96	7.02	0.019	0.217
Glaucoma patients	70.86	5.65	5.04	6.20		

LHON = Leber hereditary optic neuropathy.

TABLE III - MEANS AND STANDARD DEVIATIONS OF INTEROCULAR DIFFERENCES (IOD) IN LHON CARRIERS VS THOSE OF COMPARABLY MEASURED NORMAL CONTROLS

	Budenz (13)		LHON carriers		p value comparing means	p value comparing SDs
	Mean IOD	SD IOD	Mean IOD	SD IOD		
No.	108		50			
Average	1.3	4.7	1.6	5.6	0.726	0.061
Temporal	1.6	8.9	1.9	9.5	0.847	0.295
Superior	–0.6	13.1	0.5	11.3	0.609	0.126
Nasal	4.5	12.5	4.6	13.5	0.964	0.244
Inferior	–0.3	11.5	–0.8	12.4	0.805	0.256

LHON = Leber hereditary optic neuropathy.

tion of RGCs that leads to visual loss in LHON. In both situations, these changes remain subclinical and may represent the origin of the visual alterations that have been detected by means of contrast sensitivity and visual field analysis in LHON carriers (5, 6). Alternatively, increased variability of RNFL thickness may be influenced by the fact that the Stratus OCT algorithm incorporates blood vessels in RNFL thickness measurements. In fact, Hood and colleagues pointed out that blood vessels may contribute to the OCT RNFL thickness measurements (15). Microangiopathy is a well-known feature of LHON carriers and blood vessels caliber may be reflected by changes in RNFL thickness (16).

While LHON carriers showed higher longitudinal variability in RNFL thickness measurements with respect to the control group, they did not show higher variability when compared to glaucoma subjects, in whom the greater variability of RNFL thickness may be related to technical and physiologic reasons. The same technical issue seen in glaucoma by which blood vessels are known to affect OCT RNFL measurements may apply to LHON carriers (15, 17). Conversely, 2 additional potential sources of variability do not play a role in our study groups: 1) RNFL thicknesses in eyes of LHON carriers and controls are greater than in glaucoma patients; however, a previous study did not find any relationship between test-retest variability of OCT RNFL measurements and RNFL thickness (9). 2) While repeated measures of LHON carriers were performed annually, repeated measures of glaucoma patients and controls were performed over a much shorter period. Since no systematic changes in the means of LHON carrier RNFL thickness measurements occurred over time, calibration or operator issues are unlikely to explain the increased variability compared to controls.

From a physiologic point of view, Aydin et al, who found higher RNFL thickness values in a retrospective study of OCT before and after filtering surgery, speculated that the increase in RNFL thickness may reflect the recovery of the compressed fibers, which regain their original shape and size because of the intraocular pressure (IOP) reduction (18). Restoration of normal axoplasmic flow to the RNFL (resulting in a thicker measurement after a reduction in IOP) has been reported as an alternative explanation (19).

Accordingly, IOP decrease has been shown to induce visual field improvement, reversal of glaucomatous cupping, and RNFL thickening as measured by scanning laser polarimetry (20, 21). In contrast, a more recent investigation performed prospectively with the same technology of the present study did not confirm these findings, as the RNFL thickness did not change after large magnitude IOP reduction by medical or surgical therapy (19). Similar results have been reported also by other authors assessing RNFL thickness by means of different technologies, such as an optic nerve analyzer (Rodenstock, Munich, Germany) and confocal scanning ophthalmoscopy (HRT, Heidelberg, Germany) (22, 23). Hence, a clear physiologic explanation is still lacking.

The variability of RNFL in glaucoma may be another indication of parallels with LHON, although caution is needed because of the different age at presentation and time course. Indeed, there is mounting evidence suggesting that mitochondrial dysfunction may contribute to retinal ganglion cell death as part of glaucoma pathogenesis (24). Certainly, LHON involves mitochondrial dysfunction. Therefore, it should come as no surprise that metabolic variations can lead to the waxing and waning of pathologic expression of this disease. In other mitochondrial disorders such as mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes or Leigh diseases, oscillations of the metabolic defect/compensation are typical and well documented (25). Thus, we suggest that the most parsimonious explanation for our findings in LHON carriers is a see-sawing between the metabolic injury produced by complex I dysfunction and the compensatory response by the RGCs and their axons.

Our study has some limitations and further investigation is warranted. First, we did not evaluate the influence of the ONH size on RNFL thickness: given the likely protective effect of large ONHs on LHON clinical course, it could be conceivable that eyes with larger ONHs have a different variability in RNFL thickness measurements when compared to eyes with smaller ONHs (3). Second, the range of age in the LHON carriers sample was wider than in the control group, although no statistically significant difference was detected between the mean values. The inclusion of some patients younger than 10 in the LHON carriers sample might have contributed to the higher RNFL thickness variability in this group, due to the incomplete development of their visual system. Future studies will address this issue.

In conclusion, asymptomatic LHON carriers showed a higher variability in RNFL thickness measurements by OCT than controls. Such variability may be related to dynamic changes in RGCs and their axons due to mitochondrial dysfunction.

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