Progressive Degeneration of the Retinal Nerve Fiber Layer in Patients with Multiple Sclerosis

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Purpose. To quantify changes in the retinal nerve fiber layer (RNFL) of patients with multiple sclerosis (MS) over 3 years and to evaluate whether treatment protects against RNFL degeneration.

Methods. Ninety-four MS patients and 50 healthy subjects were followed-up over 3 years. All subjects underwent a complete ophthalmic examination, which included assessment of visual acuity (Snellen chart), color vision (Ishihara pseudoisochromatic plates), visual field examination, optical coherence tomography (OCT), and visual evoked potentials (VEPs). All patients were reevaluated at 12, 24, and 36 months to quantify changes in the RNFL.

Results. Changes were detected in RNFL thickness at the 36-month follow-up. Significant decreases ($P < 0.05$, *t*-test) were observed in the mean, superior, inferior, and temporal RNFL thicknesses, and macular volume provided by OCT, and in the P100 latency of VEP of the MS group, but only in the mean and inferior RNFL thicknesses of the healthy control group. Greater changes in the superior and inferior RNFL thicknesses during follow-up were detected in the MS group. Differences between treatments were not detected, but untreated patients had higher degeneration in the mean and superior RNFL thicknesses during the follow-up ($P = 0.040$ and $P = 0.19$, respectively).

Conclusions. Progressive axonal loss can be detected in the optic nerve fiber layer of MS patients. Analysis of the RNFL by OCT can be useful for evaluating MS progression and efficacy of treatment as a neuroprotective factor against axonal degeneration. (*Invest Ophthalmol Vis Sci.* 2012;53:8344–8349) DOI:10.1167/iovs.12-10362

Multiple sclerosis (MS) is a neurodegenerative disease characterized by axonal injury in the central nervous system (CNS), leading to progressive neurologic deficits. Evidence indicates that axonal damage occurs already in the early stages of the disease, not related to inflammatory or autoimmune episodes against myelin, and axonal degeneration is directly related to permanent functional disability.[1,2] Axonal damage in patients with MS can be detected and quantified at the level of the retinal nerve fiber layer (RNFL) using ocular imaging technologies, such as optical coherence tomography (OCT).[3–8]

The retina is a part of the CNS that is easily accessible to clinical examination. The RNFL comprises mainly nonmyelinated axons of retinal ganglion cells, so RNFL thickness measurements provide a relatively direct assessment of the axons and axonal damage.

Technologies for digital image analysis in ophthalmology, such as OCT, include the development of parameters to provide quantitative, objective, and reproducible RNFL measurements. Some studies have demonstrated RNFL loss in eyes of MS patients without optic neuritis antecedent.[3–7]

Numerous investigators have suggested the importance of examining RNFL thickness with OCT as a biologic marker of axonal damage in patients with MS,[9–14] and as a useful method for monitoring MS progression.[6,7] Some authors have even suggested that OCT could substitute for magnetic resonance imaging as a method for monitoring the disease.[8,14,15] Long-term follow-up studies are needed, however, to evaluate this hypothesis.

Fourier-domain optical coherence tomography (FD-OCT) uses a spectrometer consisting of transmission grating and an air-spaced focusing lens. In FD-OCT, depth information is acquired by analysis of the interference patterns in a spectrum of mixed reflected lights.[16] To achieve ultrahigh resolution images, time-domain (TD) OCT requires a longer acquisition time, but FD-OCT obtains 2- to 3-mm axial resolution images without a remarkable increase in the acquisition time.[17]

The signal-to-noise ratio can be further reduced with the pulsed illumination that is used by FD-OCT instead of the continuous wave illumination used in TD-OCT. The pulsed illumination reduces the detrimental effects of sample motion during scanning, so fewer artifacts and clearer images are acquired.[18]

Some investigators have used high-definition OCT to evaluate MS patients in cross-sectional studies,[19] but very few longitudinal studies using FD-OCT[20,21] have been published. These studies did not incorporate an exhaustive neuro-ophthalmologic examination of changes in visual evoked potentials (VEPs) over time. The purpose of this study was to evaluate structural and functional changes of the RNFL and correlations between these RNFL changes and disease progression or severity over a period of 3 years.

Methods

We performed a prospective longitudinal study with a duration of 3 years. In all, 188 eyes of 94 MS patients and 50 healthy subjects were evaluated at baseline, and at 12, 24, and 36 months. The study was

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approved by the hospital ethics committee and all patients signed an informed consent form detailing the purpose of this study and the tests included in the exploratory protocol, as well as the ability to stop participating in the study at any time they desired.

The study inclusion criteria were: confirmed diagnosis of MS by a neurologist based on the Poser criteria,[22] visual acuity ≥ 0.1 with the Snellen scale, and applanation intraocular pressure < 20 mm Hg. Patients with active MS outbreaks (of any neurologic deficit, not only optic neuritis) in the 6 months preceding enrollment in the study or during follow-up were excluded from the study. The reason for the study was to evaluate axonal damage secondary to the progression of MS reflecting chronic MS neurodegeneration, and acute losses that appear in times of outbreaks were not included. Patients with refractive errors > 5 diopters (D) of equivalent spherical or 3 D of astigmatism were also excluded from the study.

All subjects underwent a complete ophthalmic examination that included assessment of best-corrected visual acuity (BCVA; Snellen chart), color vision (Ishihara pseudoisochromatic plates), ocular motility, pupillary reflexes, anterior segment exam, applanation intraocular pressure (Goldmann), papillary morphology with fundoscopic exam, visual field examination, OCT, and VEP. Each eye was considered separately. All subjects were reevaluated at 12, 24, and 36 months to quantify the changes in the RNFL and assess correlations with other associated factors, such as functional disability progression measured using the EDSS (Expanded Disability Status Scale).

The neurologic variables assessed were MS phenotype (relapsing–remitting, primary progressive, and secondary progressive), disease duration, EDSS, and EDSS Fvi (visual system subset of the EDSS). Age and sex were included as covariates in the analyses. Visual acuity was measured with Snellen charts, monocular, and with a viewing distance of 6 meters. Visual field examination was performed using a Humphrey field analyzer (Carl Zeiss Meditec, Dublin, CA) using the SITA Standard 30-2 program and mean deviation was evaluated. The fundoscopy study was based on observation of the papilla morphology (normal, diffuse, or sectorial atrophy or edema) with a 78-D lens. Optical coherence tomography with a spectral-domain (SD) OCT system (Cirrus OCT 3000; Carl Zeiss Meditec) used the following protocols for retinal imaging: fast RNFL thickness (3.4-mm circular scans) and fast macula. Parameters evaluated were mean thickness, RNFL thicknesses of the four quadrants, and macular volume. VEP analysis was performed using commercial software that facilitates the acquisition and analysis of auditory, visual, and somatosensory evoked potentials (Neuronic Sense Witness System 4.0 device; IC Neuronic S.L.; Zaragoza, Spain). Silver chloride–plated disk electrodes were placed on the scalp at the occipital (Oz, active electrode) and frontal (Fz, reference electrode) areas. The frequency of the stimulation pattern was 2 Hz. Mean luminance was 93.5 candelas/m^2, with a contrast of 99%. The latency and amplitude of the positive fundamental component (P100) were analyzed.[23]

All variables were registered in a database created with a commercial database application program (FileMaker Pro 8.5; FileMaker, Inc., Santa Clara, CA). Two statistical analyses were performed: first, an observational cross-sectional study using baseline measurements, and then a longitudinal study. In the cross-sectional analysis, the independent variables were MS phenotype and immunomodulatory therapy, and the dependent variables were the parameters provided by the different techniques included in the study protocol. Modifier variables were age, sex, and intraocular pressure. All subjects were revaluated after 12, 24, and 36 months from baseline, and the longitudinal analysis was performed.

Statistical analysis was performed using commercial predictive analytics software (SPSS, version 19.0; SPSS, Inc., Chicago, IL). The normality of the sample distribution was confirmed using the Kolmogorov–Smirnov test. Values of $P < 0.05$ were considered statistically significant. Results of each variable in subsequent visits were compared for both groups (MS and healthy controls) by ANOVA, to detect differences in RNFL associated with disease progression. Pearson's correlation test was used to correlate changes in structural (OCT) and functional (visual field, BCVA, chromatic vision, and VEP) variables, and to correlate measurements registered during the study on the disability status of the MS patients (EDSS). Correlation analysis was used to determine the Pearson correlation coefficient (r) and the statistical significance of the association (P). The Pearson correlation coefficient (r) can range between +1 and −1: a value of 0 indicates that there is no association between the two variables. A value > 0 indicates a positive association, that is, as the value of one variable increases so does the value of the other variable. A value < 0 indicates a negative association, that is, as the value of one variable increases the value of the other variable decreases.

Finally, patients were divided into two groups: patients with treatment for MS and patients without treatment. Patients treated with mitoxantrone were excluded to minimize bias. A statistical comparison between the groups was performed to analyze whether treatment is a protective factor against RNFL degeneration in patients with MS.

Results

The MS group included 64 females and 30 males with a female/male ratio of 3:2. The control group included 17 females and 33 males (ratio of 3:2). Age and sex in the MS and control groups were not significantly different ($P = 0.568$ and 0.394, respectively). Eighty-six patients (92.6%) belonged to the relapsing–remitting MS group, 6 patients (6.4%) to the secondary progressive group, and 1 patient (1.1%) to the

Table 1. Functional and Structural Measurements Obtained at Baseline and Annual Examinations in Patients with Multiple Sclerosis (at 1, 2, and 3 Years of Follow-Up)

	Baseline	1 Year	2 Years	3 Years	Annual Change	*P*
BCVA	0.91 (0.24)	0.94 (0.21)	0.92 (0.11)	0.90 (0.34)	−0.003	**0.034**
Ishihara test	18.06 (3.04)	17.93 (3.22)	18.12 (3.76)	18.08 (3.23)	0.03	0.278
Visual field, MD (dB)	−3.08 (2.44)	−3.01 (3.23)	−3.62 (2.14)	−3.87 (2.76)	−0.42	0.689
OCT mean thickness	89.43 (10.65)	86.95 (11.28)	84.50 (10.62)	81.03 (10.65)	−3.67	**0.004**
OCT superior thickness	115.78 (15.03)	114.26 (16.23)	109.45 (14.79)	105.01 (13.45)	−4.98	**0.005**
OCT nasal thickness	69.06 (17.47)	68.12 (17.08)	67.57 (16.16)	66.99 (15.89)	−1.12	0.061
OCT inferior thickness	113.67 (14.56)	109.30 (16.34)	105.30 (19.22)	101.12 (15.59)	−4.88	**0.008**
OCT temporal thickness	59.61 (13.45)	56.57 (18.97)	55.91 (15.63)	52.78 (15.11)	−3.61	**0.019**
OCT macular volume	6.57 (0.43)	6.43 (0.23)	6.42 (0.39)	6.43 (0.34)	−0.18	**0.031**
VEP amplitude, mV	11.09 (2.99)	11.05 (5.77)	11.12 (5.27)	10.99 (4.57)	−0.04	0.443
VEP latency, ms	124.59 (11.32)	118.45 (6.45)	116.17 (5.54)	114.16 (7.31)	0.40	**0.039**

Results reported as mean and SD in parentheses; mean of annual change in each parameter over the 3-year follow-up and significance level (ANOVA) comparing changes between visits in longitudinal analysis. Significant measurements are indicated in bold font ($P < 0.05$). RNFL thickness is measured in micrometers (μm), and macular volume is measured in mm^3.

Table 2. Functional and Structural Measurements Obtained in the Healthy Control Group at Baseline and Annual Examinations (at 1, 2, and 3 Years of Follow-Up)

	Baseline	1 Year	2 Years	3 Years	Annual Change	*P*
BCVA	0.95 (0.14)	0.95 (0.18)	0.95 (0.17)	0.95 (0.16)	<−0.001	0.876
Ishihara test	19.65 (1.34)	19.64 (1.78)	19.66 (1.66)	19.63 (1.82)	−0.001	0.573
Visual field, MD (dB)	−0.77 (2.10)	−0.78 (1.98)	−0.75 (1.61)	−0.75 (1.55)	−0.008	0.770
OCT mean thickness	95.97 (11.60)	94.72 (11.82)	93.02 (10.62)	91.13 (10.65)	−0.152	**0.045**
OCT superior thickness	117.95 (15.61)	116.34 (15.71)	115.45 (14.39)	115.04 (13.45)	−0.079	0.245
OCT nasal thickness	72.53 (9.59)	72.27 (10.02)	72.00 (10.34)	71.66 (11.70)	−0.022	0.156
OCT inferior thickness	126.25 (13.34)	125.87 (13.24)	124.03 (14.29)	123.19 (13.33)	−0.180	**0.032**
OCT temporal thickness	68.53 (11.90)	67.96 (11.39)	67.56 (15.03)	67.25 (15.11)	−0.042	0.441
OCT macular volume	7.11 (0.34)	7.10 (0.28)	7.11 (0.21)	7.10 (0.20)	−0.005	0.398
VEP amplitude, mV	14.55 (2.53)	14.56 (2.77)	14.54 (3.34)	14.54 (3.67)	<−0.001	0.689
VEP latency, ms	100.32 (5.92)	100.11 (4.65)	100.43 (5.01)	110.41 (6.88)	0.003	0.220

Results are reported as mean and SD in parentheses; mean of annual change in each parameter over the 3-year follow-up and significance level (ANOVA) comparing changes between visits in longitudinal analysis. Significant measurements are indicated in bold font ($P < 0.05$). RNFL thickness is measured in micrometers (μm), and macular volume is measured in mm^3.

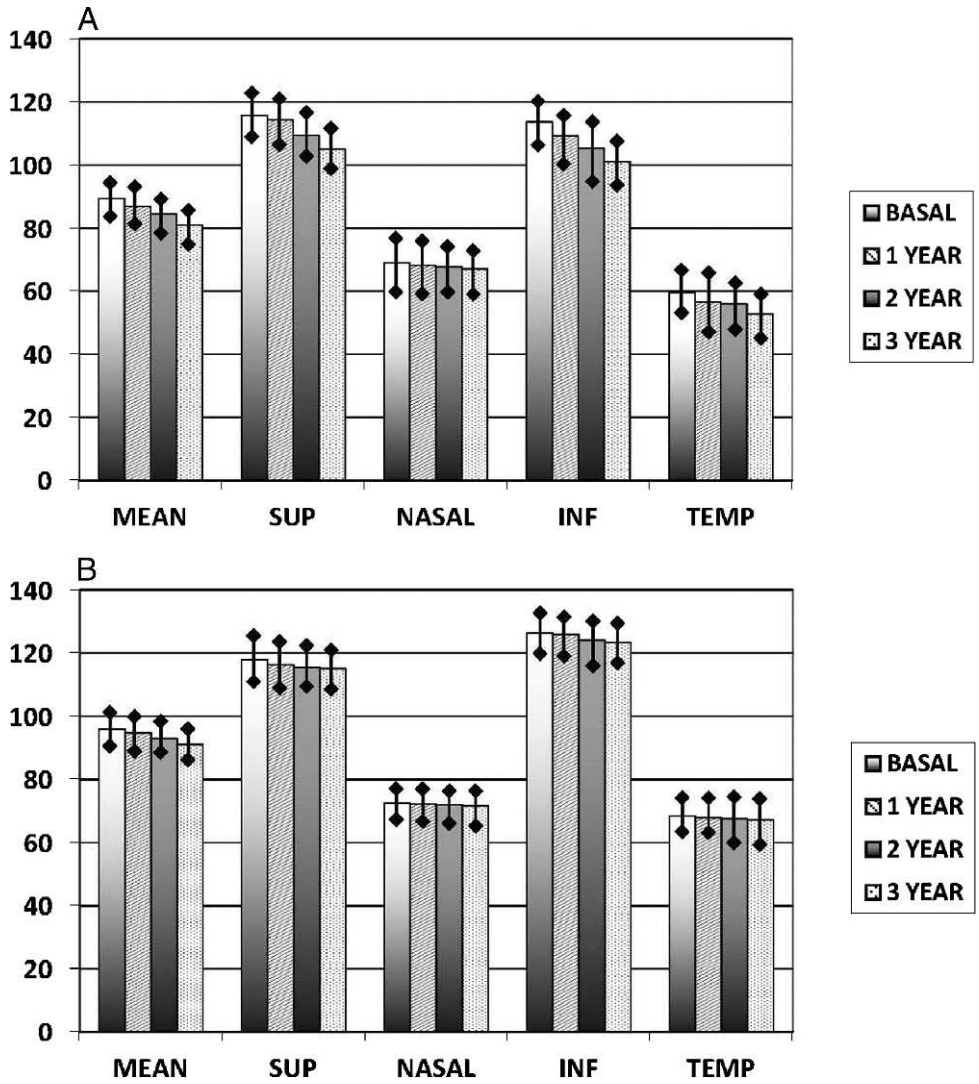

Figure 1. (**A**) Mean RNFL thickness measured by OCT in patients with multiple sclerosis at baseline examination and the revisions made in the first, second, and third years. Thickness of the RNFL is measured in micrometers (μm). (**B**) Mean RNFL thickness measured by OCT in healthy controls at baseline examination and the revisions made in the first, second, and third years. SUP, superior; INF, inferior; TEMP, temporal.

Table 3. Differences in Mean Change in Each Variable during the 3-Year Follow-Up for Two Groups: Treated and Untreated Patients

	Change at 3 Years in Untreated Patients	Change at 3 Years in Treated Patients	*P*
Functional variables			
BCVA (Snellen)	−0.003 (0.001)	−0.002 (0.001)	0.257
Ishihara test	0.02 (0.002)	0.04 (0.001)	0.487
Visual field MD (dB)	−0.22 (0.10)	−0.49 (0.07)	0.312
EDSS	0.10 (0.01)	−0.10 (0.01)	**0.034**
EDSS Fvi	0.03 (0.002)	−0.01 (0.001)	0.457
RNFL thickness (OCT)			
Mean, μm	−3.67 (0.72)	−3.39 (0.66)	**0.040**
Superior, μm	−4.98 (0.54)	−3.97 (0.72)	**0.019**
Nasal, μm	−1.12 (0.52)	−1.21 (0.38)	0.298
Inferior, μm	−4.88 (0.91)	−4.61 (0.80)	0.389
Temporal, μm	−3.61 (0.83)	−3.60 (0.91)	0.053
Macular volume, mm^3	−0.18 (0.02)	−0.15 (0.02)	0.076
VEP			
Latency, ms	0.05 (0.01)	0.05 (0.01)	0.770
Amplitude, mV	-0.41 (0.01)	−0.37 (0.01)	0.523

Differences in mean change of each parameter during the 3-year follow-up and SD in parentheses, for the two groups: treated and untreated. Significance difference in change between treated and untreated groups based on Student's *t*-test.

primary progressive group. Antecedents of diplopia were present in 56 patients (29.8%) and neuritis in 45 patients (23.9%). The mean EDSS score at baseline was 2.5 (range: 0–8).

With respect to the percentage of patients assigned to each treatment group, the largest group comprised subjects who received no treatment (36%). Among treated patients, the largest group received beta interferon 1b (Betaferon; Bayer HealthCare, Leverkusen, Germany) (21.3%), followed by beta interferon 1a (Rebif; EMD Serono, Inc., Rockland, MA [16.0%] or Avonex; Biogen Idec International GmbH, Zug, Switzerland [12.8%]), glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd., Petach Tikva, Israel) (11.7%), and mitoxantrone (Mitoxantrona; Beijing Mesochem Technology Co., Ltd., Beijing, China) (2.0%).

Results of functional and structural parameters evaluated and significant differences between the baseline and 3-year examinations are shown in Tables 1 and 2. In MS patients, we detected a significant loss in BCVA; mean, superior, inferior, temporal RNFL thicknesses, and macular volume obtained with OCT; and a significant delay in the P100 wave latency in VEP (Table 1 and Fig. 1A). In healthy subjects, we observed a significant loss only in the mean and inferior RNFL thicknesses (annual changes of −0.152 and −0.180 μm, respectively; $P = 0.045$ and 0.032; Table 2 and Fig. 1B).

In the MS group, annual mean RNFL thickness measured by OCT showed a clear decrease over 3 years (Fig. 1A). The parameters with the most changes between baseline and the 3-year follow-up in MS patients were the superior and inferior RNFL thicknesses, with a decrease in annual mean thickness of 4.98 ($P = 0.015$) and 4.88 μm, respectively ($P < 0.001$).

Correlation analysis was performed to determine the association between the changes registered in parameters during the 3-year follow-up. The changes in structural variables during the follow-up provided by OCT were not significantly correlated with the changes in the functional variables during these 3 years (BCVA, chromatic vision, VEP, EDSS, and EDSS Fvi); however, there was a moderate positive correlation between the change registered during the 3-year follow-up in the mean deviation of the visual field and changes in mean ($r = 0.407$, $P = 0.034$) and temporal ($r = 0.521$, $P = 0.029$) RNFL thicknesses during the 3 years of the longitudinal study.

Changes in the functional and structural parameters over the 3-year follow-up were compared between treated and nontreated patients. Untreated patients showed a higher loss of thickness over the 3-year follow-up in all RNFL parameters (except in the nasal quadrant), but statistical differences were detected only in the mean and superior RNFL thicknesses (Table 3, Fig. 2).

Discussion

In the present study, we quantified changes in the RNFL over a 3-year period to analyze the ability of RNFL evaluation to be used as a biomarker of neurodegeneration and disability in patients with MS, and to compare axonal degeneration

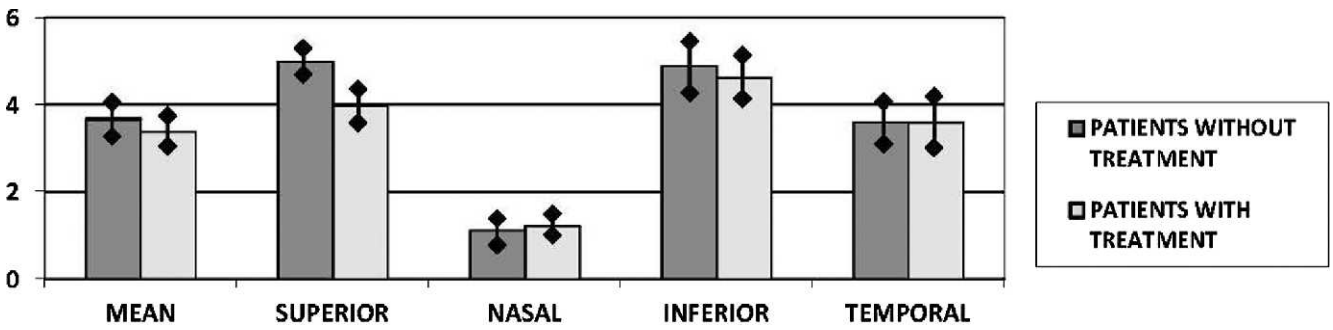

Figure 2. Mean decrease in RNFL thickness (measured in μm) over the 3-year follow-up in treated and nontreated groups.

between treated and untreated patients. Numerous studies have demonstrated RNFL thinning not only in eyes with a previous episode of optic neuritis, but also in patients with MS who have never had an acute clinical episode of optic neuritis.[12,24,25] Measurements of RNFL thickness provided by digital imaging devices such as OCT have been suggested to be useful as an indirect marker of brain atrophy in MS.[15,26,27] In addition to RNFL thickness, OCT can also measure macular volume. The macula is composed of ganglion cell bodies, so macular volume may be a useful measurement of axonal loss (based on RNFL thickness) associated with neuronal degeneration.[28]

The new SD-OCT has advantages over previous TD instruments. SD-OCT provided axial resolutions of approximately 5 to 7 μm (compared with Stratus TD-OCT at 10 μm),[29] and the measurements provided by SD devices (such as Cirrus OCT) have better intraobserver and interobserver reproducibility.[8] In addition, several studies suggest that SD technology detects more retinal and RNFL conditions than conventional TD technology.[30,31]

We analyzed each eye separately because each eye can be affected differentially, especially in patients who present with unilateral episodes of optic neuritis. In addition, the OCT devices interpret the three-dimensional profile of the RNFL thickness in each eye and compare it separately with the normative base. Finally, a macular volume reduction in MS patients has been correlated with neuronal necrosis, demyelination, and axonal damage. The loss of ganglion cells also leads to a reduction of the macular volume (independently in each eye).[32–40] Some authors, however, consider the inclusion of only one eye of each patient adequate for statistical analysis because RNFL measurements correlate significantly between the two eyes.

The P100 wave latency of VEP is a parameter with high diagnostic ability in MS.[41,42] The P100 latency is delayed after an optic neuritis episode and this delay is maintained for years.[43] We observed an increase in the latency over the 3-year follow-up period due to the loss of visual pathway function in patients with MS disease progression. Nonreduction of VEP amplitude despite the reduction of the OCT RNFL can be caused by the remaining macular volume. Eighty percent of the VEP amplitude depends on the macula response and, therefore, does not involve the RNFL of the arcuate area. We also observed a nonsignificant increase in the P100 latency in the control group, which may be due to the variability of the device. Our findings reveal that a reduction in the mean or temporal RNFL thickness is associated with a decrease in the mean deviation of the visual field.

Only a few authors have examined the effects of MS treatments on RNFL degeneration. Garcia-Martin et al.[44] concluded that treatment might be a protective factor against RNFL loss associated with disease progression. In the present study, we observed that the treatment for MS has a protective effect on the loss of RNFL thickness, but there were no differences between different types of treatments. MS is a chronic degenerative disease in which axonal loss occurs slowly and gradually in the absence of active inflammatory outbreaks, so a 3-year follow-up is a short time to detect small RNFL modifications in a population such as ours, which included only stable patients who had no acute relapsing MS episodes. Longer studies with a larger sample are needed to assess whether any of the currently approved treatments for MS have a significant protective effect on axonal loss detected by RNFL analysis.

Based on other studies, 6 months is the time required for measurements made with digital image analysis techniques to record retrograde degeneration after an inflammatory episode in the optic nerve.[32,33] In the present study, patients with optic neuritis in the 6 months preceding the study were excluded, so all subjects were considered to have stable MS. If patients with active acute relapsing MS episodes had been included, higher differences between treatments may have been detected. The reason for excluding patients with acute relapsing MS episodes was to assess only axonal damage secondary to the progression of MS disease. The RNFL changes registered in our study were caused only by MS-related chronic neurodegeneration, not by acute heavy axonal loss that occurs in acute relapsing MS episodes.

In conclusion, analysis of RNFL measured by OCT has good capability for detecting axonal damage in MS patients, and can be used to evaluate disease progression and effectiveness of the various therapies currently in use.

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