Original article

Atrophy of the retinal nerve fibre layer in multiple sclerosis patients. Prospective study with two years follow-up

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ABSTRACT

Objective: To evaluate the changes over two years in the retinal nerve fibre layer (RNFL) of patients with multiple sclerosis (MS). To compare the ability of optical coherence tomography (OCT), scanning laser polarimetry (GDx), visual evoked potentials (VEP) and visual field examination to detect axonal loss in these patients.

Material and methods: Fifty eyes of MS patients without episodes or optic neuritis during follow-up were enrolled in this study. All patients underwent a complete ophthalmic examination that included visual acuity (VA), colour vision, refractive evaluation, visual field examination, OCT, GDx and VEP. All the patients were re-evaluated over a period of 12 and 24 months. Correlations between parameters were analysed by Pearson’s test.

Results: There were changes in the RNFL thickness in MS patients with a 12 and 24-month follow-up. Differences between baseline and 2-year evaluation were statistically significant \( (p \leq 0.05, \ t \text{ test}) \) in the mean, superior and inferior RNFL thickness and macular volume provided by OCT, while no significant differences were found using functional parameters (VA, colour vision, visual field and VEP) and GDx. The greater differences were obtained in the inferior RNFL thickness (113.67 frente a 105.39 \( \mu \text{m}, p < 0.001 \)). Correlations were observed between structural parameters using GDx and TCO.

Conclusions: Progressive axonal loss can be detected in the optic nerve of MS patients. Measurements provided by TCO are useful tools to evaluate structural abnormalities in the RNFL and changes in macular volume, however these changes were not detected using functional tests or GDx.

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Introduction

Multiple sclerosis (MS) is a chronic disease which mainly affects young people and can cause progressive neurological deficits and considerable functional disability. One of the main causes of MS is visual deficit, which appears in 80% of patients and generally expresses as loss of visual acuity (VA) or as an alteration in ocular motility. In about half of patients, MS onset begins with visual alterations.1,2

The most widely accepted theory is that MS has a multiple pathogeny associated inflammation, and demyelinization and axonal damage. There is evidence that axonal damage is directly related to the permanent functional disability.3,4 Until recently, it was believed that axonal damage and disability were associated to inflammation and self-immune processes against myelin, but in the past few years several authors have demonstrated the existence of axonal damage in very early stages of the disease without relation to inflammatory or self-immune episodes against myelin.5 Several studies, some made on experimentation animals, have evidenced the existence of new axonal loss mediators.6,7 Clinical2,8 radiological9,10 and pathological11 evidence allowed to define axonal damage as the central element of the MS pathology and clinical expressions, as it influences the inflammatory response and is related to the disability.

The retina nervous fibre layer (RNFL) comprises ganglionic cell axons which join each other in the optic nerve. These axons lack myelin and therefore can be good axonal damage markers. Numerous authors have described the existence of defects in the RNFL of MS patients, both in those who exhibited optic neuritis episodes as in those who did not have ophthalmological expressions, even through the former seem to have a greater involvement of this retinal structure.12-17

A clinical assessment of the optic nerve must be approached from a functional viewpoint as well as from a structural one. The recent application of digital imagine techniques in ophthalmology have brought about the appearance and development of parameters enabling quantitative, objective and reproducible RNFL measurements. Of relevance among said imaging techniques are opti coherence tomography (OCT) and laser polarimetry (GDx).18-20 These new diagnostic instruments have allowed some authors to observe alterations in the RNFL of MS patients even without a clinical history of optic neuritis episodes,12-17 which points to the existence of axonal damage not associated to inflammation. If the usefulness of the study assessing the RNFL as a biological marker of axonal damage in MS is confirmed, it could also prove very useful in two main areas: firstly, to control the progression of the disease as axonal damage is directly related to the
functional disability secondary to MS. Secondly, it could be a good marker to assess the efficiency of disease modifying therapies which are increasingly utilized in these patients in order to diminish the progression thereof.\textsuperscript{21-24}

Studies have been developed which appear to indicate said usefulness of RNFL assessment, but prospective cohort studies are still required to determine with greater precision the relationship between the RNFL defects and overall neuronal damage as well as its parallel progression in the course of the disease.

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**Material and methods**

A total of 25 MS patients were prospectively included (50 eyes) and assessed at baseline, at 12 and 24 months.

The following criteria were applied for including subjects in the study: a) MS diagnostic confirmed by a neurologist on the basis of Posser’s criteria; b) VA F 0.1 or above in each eye to enable the adequate development of the exploration protocol, and c) applanation intra-ocular pressure values below 20 mmHg. The patients who had suffered an optic neuritis episode or other expressions of the disease considered to be a manifestation thereof within six months prior to their inclusion in the study or during the follow-up were excluded, as were those having a refraction defect above 5 diopters of spherical equivalent or 3 dioptres of astigmatism.

The study was approved by the Ethical Committee of the hospital, and all participants signed an informed consent detailing the objective of the study and the tests included in the exploration protocol as well as the option of leaving the study when they desired. All the subjects underwent a complete ophthalmological exploration which comprised VA with Snellen optotype, color test (Ishihara chromatic sheets), ocular motility, pupil reflexes, refraction defect measurement, anterior pole exploration, applanation tonometry, funduscopic assessment of the papilla, computerized perimetry utilizing the Humphrey field analyzer (Carl Zeiss Meditec, Dublin, California, USA) with the SITA Standard strategy, program 30-2, OCT, GDx and visual evoked potentials (VEP).

The optical coherence tomography (OCT) (Stratus OCT 3000. Carl Zeiss Meditec, Dublin) utilized the following protocols for obtaining retinal images: fast RNFL thickness (3.4 mm circular scans) and fast macula. The assessed parameters were the mean RNFL thickness, the thickness in each of the four papillary quadratns and the macular volume.

The laser polarimetry with variable corneal compensation (GDx VCC, Laser Diagnostic Technologies, San Diego) was performed with 3.2 mm diameter circular scans centered on the papilla in order to measure the RNFL thicknesses (nerve fiber index [NFI], temporal-superior-nasal-inferior average [TSNIT], superior average, inferior average and standard deviation TSNIT).

The VEP utilized in the study were of the pattern type recorded with the Medelec ER 94/ Sensor ST 10 equipment and disc electrodes coated with silver chloride placed on the occipital area of the scalp (Oz, active electrode) and the frontal area (Fz, reference electrode). As ground electrode, a low impedance electrode was placed on the patient forehead. The stimuli were presented in a monocular form and the alternate frequency of the pattern stimuli was of 2Hz. A video monitor of 26 x 20 cm placed at one metre of the subject was utilized (visual angle of 16\degree) and the size of the squares was 1\degree of arc. The luminance average was of 93.5 candelas/m\textdegree, with a contrast of 99\%. The latency and width of the positive fundamental component were measured, taken conventionally as a descending deflection (P100 wave).

The recorded neurologial assessment variables were age, sex, MS phenotype (relapsing-remittent, primary progressive, secondary progressive and central nervous system demyelinating disease without filiation), evolution time since diagnostic and the Expanded Disability Status Scale (EDSS). Said explorations were performed at baseline and one and two years later in order to assess changes in the recorded parameters and the correlation between said changes.

In the statistical analysis, all the above-mentioned variables were recorded in a database made with FileMaker Pro 5.0 software. A first transversal study was made in which the independent variables were the presence or absence of MS and the immunomodulating treatment, with the dependent variables being the parameters supplied by the various diagnostic techniques included in the study protocol. The effect-modifying variables included in the study were age, sex and intra-ocular pressure data. After 12 and 24 months from the first study, the patients were reassessed applying the same parameters, after which a comparative statistical analysis of the obtained results was carried out.

In addition, a correlation study was performed between the change observed in the course of the 2 years of follow-up between the variables by means of the Pearson test.

All the statistical analyses were made with the statistical software SPSS version 15.0 (SPSS, Inc., Chicago, USA). Prior to the data analysis it was observed that the majority fitted within normal values by means of the Kolmogorov-Smirnov test. The results obtained from each variable in both explorations were compared with the t for Student parametric test.

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**Results**

The study include 19 males and 31 females, with the male/female ratio being approximately 2:3. The mean age was 42.21 years (range: 20-69 years) and the mean evolution time of the disease since diagnostic was of 8.97 years (range: 0.5-26 years). The predominant MS type was relapsing-remittent, with 21 patients (88\%) against the other types. One patient exhibited primary progressive MS (6\%) and another one had secondary progressive MS (6\%). The mean on the EDSS scale utilized to assess the patients was of 2.66 (range: 0-6.5) in the baseline evaluation and of 2.86 (range: 0-6.5) in the check-up 2 years later.

The results of all the parameters assessed in this study at baseline and in the two annual assessment, as well as the difference between both explorations and the significance level of the comparison are detailed in table 1. As said table
illustrates, significant differences were identified between the baseline evaluation and 2 years year in the mean, superior and inferior RNFL thickness and in the macular volume obtained with OCT. However, the rest of parameters exhibited a clear trend towards a reduction in the RNFL without said changes being statistically significant. The NFI value of the GDx exhibited an increase during the follow-up period, which translates into a reduction of the RNFL because this index represents an alteration of the integrity of this structure, as higher values indicate greater alteration of the RNFL thickness. This parameter was inferior at baseline than at the 2-year assessment, although not statistically significant. The same occurred with the VEP latency values, which showed an increase throughout the follow-up, which also reflects the loss of functionality of the visual pathway together with an increase in its value.

The parameter which most changed during the follow-up was the inferior RNFL thickness measured in the OCT, which diminished on average 8.28 µm in 2 years (113.67 µm at baseline against 105.39 µm in the annual assessment), as shown in figure 1. The probability that said reduction of the mean RNFL thickness in the course of 2 years in MS patients could be random is smaller than 0.1% (p<0.001). The superior quadrant and the mean thickness measured by OCT also exhibited significant differences, with a mean reduction of 6.47 and 4.92 µm respectively during the follow-up period. Similarly, the macular volume diminished significantly in these patients, as shown in figure 2.

The correlation study between the various parameters was made with a Pearson correlation test which did not evidence correlation between the differences observed in the structural variables and the differences in VA or in the VEP. However, there was a significant correlation (p<0.5) between the differences observed in the mean deviation of the visual field

![Figure 1 – Graphic representation with box diagrams showing the mean thickness of the retina nervous fibre layer measured with Optic Computerized Tomography (OCT) in the baseline exploration, at month 12 and 24 of follow-up in multiple sclerosis patients.](image-url)
in the baseline, 12-month and 24-month explorations in changes measured with optic coherence tomography (OCT).

**Figure 2 – Box diagrams illustrating macular volume changes measured with optic coherence tomography (OCT) in the baseline, 12-month and 24-month explorations in multiple sclerosis patients.**

and some OCT parameters and between the various structural variables amongst themselves, as can be seen in table 2.

**Discussion**

The objective of the study was to quantify RNFL defects in MS patients and to observe whether, after 12 and 24 months, they evidenced a loss of fibres above that which physiologically takes place with age. MS is a progressive neurodegenerative disorder which affects the central nervous system and is therefore considered to cause RNFL reductions. Many authors consider that this neuronal loss takes place even without inflammatory episodes or outbreaks of the disease. Our study included only patients who did not exhibit activity during the follow-up period both at the ophthalmological and systemic level, with the aim of evaluating and quantifying the RNFL associated to the progression of MS.

Typically, ophthalmoscopy and single-color retinal photographs were utilized to obtain a qualitative and subjective assessment of the RNFL and the optic nerve. This was able to detect only defects with losses above 50% of the retina ganglionary cells. At present, the application of digital imaging techniques such as OCT and GDx allow a more precise assessment of the RNFL. These techniques, which are not invasive and hardly dependent on the examiner, are able to provide very precise analyses and quantitative measures in a few minutes without causing discomfort to the patient.

The visual function in MS has been researched by various authors and a visual involvement has been demonstrated to exist in these patients, particularly those who exhibited previous optic neuritis episodes. This visual dysfunction has been observed in VA as in perimetry, in color vision and in the VEP, which show longer latencies and smaller width in MS patients. However, to date only transversal studies have been published, comparing the visual function between MS patients and healthy subjects, but the progression in time of both groups has not been researched. In our study we observe a tendency towards an increase of visual dysfunction in MS patients through the follow-up period, although the differences are not statistically significant. For this reason, the RNFL structural tests seem more sensitive to detect alterations caused by MS in the visual pathway than the evaluation of its visual function.

This study has seen a reduction in the RNFL thickness of MS patients from one year to the other. A longitudinal study of 187 eyes of healthy patients assessed by means of OCT the physiological loss of retina fibres that occurs with age and determined that in a normal person the average RNFL diminishes 0.16 µm per year. This loss is more acute in people over 50 years of age. The results of our study show that MS patients have a loss of 2.46 µm per year in the mean RNFL thickness, which represents fifteen times the reduction suffered by healthy individuals in this retinal structure. This leads us to consider the existence of physiopathological mechanisms other than optic nerve inflammation that cause axonal damage in this disease.

Patients who suffered optic neuritis within 6 months prior to the beginning of the study were excluded following the criterion of most authors, who regard six months as the necessary period for the digital imaging technologies like OCT and GDx to measure the retrograde degeneration which takes place after an inflammatory episode of the optic nerve, particularly in the case of retrobulbar neuritis.

Among the various RNFL measurement technologies, OCT stood out as the most useful for diagnostic and follow-up of optic neuropathies and MS patients. This fact has led some authors to suggest that OCT could be useful to monitor the progression of the disease and the response to treatment in clinical trials, although it is difficult to determine if it is correct to extrapolate neuronal changes observed in the RNFL to the rest of the central nervous system because this structure represents a small part thereof. In addition, MS is a multifocal disease which can affect different areas of the central nervous system in highly variable ways. It should also be considered that the functional as well as structural tests involve a degree of variability which could account for some of the changes observed in the measurements taken throughout the study and that the quality of the images obtained with the digital image analysis techniques should be high for the data to be reliable. This is not always possible, particularly in patients who do not have adequate visual fixation, have ocular motility disorders, nistagmus or other alterations hindering the execution of the tests. These issues should be taken into account in the interpretation of the RNFL assessment results and explain the need to carry out new studies which assess the prognostic value of RNFL analysis in MS patients.

To conclude, we can state that the evolution of MS causes axonal damage at the level of the optic nerve that is greater than the damage experienced physiologically by the healthy population, even though the patient did not exhibit symptoms of activity of outbreaks of the disease. In addition, said axonal degeneration should be measurable by means of OCT.
Table 2 – Correlations observed between the differences of each parameter at baseline and 12-month explorations

<table>
<thead>
<tr>
<th>Difference of exploration in 2nd year minus baseline</th>
<th>VA</th>
<th>DM</th>
<th>OCT medium</th>
<th>OCT superior</th>
<th>OCT nasal</th>
<th>OCT inferior</th>
<th>OCT temporal</th>
<th>OCT macular volume</th>
<th>GDx NFI</th>
<th>GDx Average TSNIT</th>
<th>GDx Average superior</th>
<th>GDx Average A TSNIT</th>
<th>GDx Desv TSNIT</th>
<th>VEP Amplitude</th>
<th>VEP Latency</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA</td>
<td>–</td>
<td>–0.083</td>
<td>0.122</td>
<td>–0.245</td>
<td>0.223</td>
<td><strong>0.382</strong></td>
<td>0.014</td>
<td>0.251</td>
<td>–0.131</td>
<td>0.248</td>
<td>0.239</td>
<td>–0.002</td>
<td>0.027</td>
<td>–0.095</td>
<td>0.043</td>
</tr>
<tr>
<td>MD</td>
<td>–0.083</td>
<td>–</td>
<td><strong>0.386</strong></td>
<td><strong>0.407</strong></td>
<td>–0.026</td>
<td>0.057</td>
<td>0.269</td>
<td>–0.002</td>
<td>–0.053</td>
<td>0.221</td>
<td>0.072</td>
<td>–0.192</td>
<td>–0.023</td>
<td>–0.693</td>
<td>–0.602</td>
</tr>
<tr>
<td>OCT medium</td>
<td>0.122</td>
<td>–0.083</td>
<td>–</td>
<td><strong>0.386</strong></td>
<td><strong>0.407</strong></td>
<td>0.057</td>
<td>0.269</td>
<td>–0.002</td>
<td>–0.053</td>
<td>0.221</td>
<td>0.072</td>
<td>–0.192</td>
<td>–0.023</td>
<td>–0.693</td>
<td>–0.602</td>
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<tr>
<td>OCT superior</td>
<td>–0.245</td>
<td>0.407</td>
<td>0.626</td>
<td>–</td>
<td>–0.057</td>
<td>–0.173</td>
<td>0.250</td>
<td>0.083</td>
<td>–0.115</td>
<td>–0.079</td>
<td>–0.263</td>
<td>0.301</td>
<td>–0.214</td>
<td>–0.029</td>
<td>–0.432</td>
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<tr>
<td>OCT nasal</td>
<td>0.223</td>
<td>–0.026</td>
<td>0.399</td>
<td>–0.057</td>
<td>–</td>
<td><strong>0.452</strong></td>
<td><strong>0.405</strong></td>
<td>–0.086</td>
<td>–0.395</td>
<td>0.215</td>
<td>0.281</td>
<td>–0.041</td>
<td>0.163</td>
<td>0.378</td>
<td>–0.309</td>
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<tr>
<td>OCT inferior</td>
<td>0.382</td>
<td>0.057</td>
<td>0.506</td>
<td>–0.173</td>
<td><strong>0.452</strong></td>
<td>–</td>
<td>–0.072</td>
<td>0.176</td>
<td>–0.138</td>
<td>0.258</td>
<td>0.036</td>
<td>0.02</td>
<td>–0.154</td>
<td>–0.172</td>
<td>–0.059</td>
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<td>OCT temporal</td>
<td>0.014</td>
<td>0.269</td>
<td>0.419</td>
<td>0.25</td>
<td>–0.405</td>
<td>–0.072</td>
<td>–</td>
<td>0.011</td>
<td>0.244</td>
<td>–0.133</td>
<td>–0.329</td>
<td>0.118</td>
<td>–0.027</td>
<td>–0.577</td>
<td>–0.171</td>
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<td>–0.002</td>
<td>0.076</td>
<td>0.083</td>
<td>–0.086</td>
<td>0.176</td>
<td>0.011</td>
<td>–</td>
<td>–0.173</td>
<td>0.452</td>
<td>0.48</td>
<td>0.175</td>
<td>0.363</td>
<td>–0.516</td>
<td>–0.654</td>
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<td>–0.053</td>
<td>–0.171</td>
<td>–0.115</td>
<td>–0.395</td>
<td>–0.138</td>
<td>0.244</td>
<td>–0.173</td>
<td>–</td>
<td><strong>-0.725</strong></td>
<td><strong>-0.728</strong></td>
<td><strong>-0.684</strong></td>
<td>–0.381</td>
<td>–0.118</td>
<td>0.122</td>
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<tr>
<td>GDx average TSNIT</td>
<td>0.248</td>
<td>0.221</td>
<td>0.064</td>
<td>–0.079</td>
<td>0.215</td>
<td>0.258</td>
<td>–0.133</td>
<td>0.452</td>
<td>–0.725</td>
<td>–</td>
<td>0.829</td>
<td>0.305</td>
<td>0.588</td>
<td>–0.684</td>
<td>–0.399</td>
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<tr>
<td>GDx average superior TSNIT</td>
<td>0.239</td>
<td>0.072</td>
<td>–0.235</td>
<td>–0.263</td>
<td>0.281</td>
<td>0.036</td>
<td>–0.329</td>
<td>0.480</td>
<td>–0.728</td>
<td><strong>0.829</strong></td>
<td>–</td>
<td>0.235</td>
<td><strong>0.803</strong></td>
<td>–0.87</td>
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<tr>
<td>GDx average inferior TSNIT</td>
<td>–0.002</td>
<td>–0.192</td>
<td>0.258</td>
<td>0.301</td>
<td>–0.041</td>
<td>0.02</td>
<td>0.118</td>
<td>0.175</td>
<td><strong>-0.684</strong></td>
<td>0.305</td>
<td>0.235</td>
<td>–</td>
<td>0.197</td>
<td>–0.306</td>
<td>–0.115</td>
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<td>GDx deviation TSNIT</td>
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<td>–0.023</td>
<td>–0.308</td>
<td>–0.214</td>
<td>–0.163</td>
<td>–0.154</td>
<td>–0.027</td>
<td>0.363</td>
<td>–0.381</td>
<td><strong>0.588</strong></td>
<td><strong>0.803</strong></td>
<td>0.197</td>
<td>–</td>
<td>–0.652</td>
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<td>VEP amplitude</td>
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<td>–0.693</td>
<td>–0.241</td>
<td>–0.432</td>
<td>0.378</td>
<td>–0.172</td>
<td>–0.577</td>
<td>–0.516</td>
<td>–0.118</td>
<td>0.684</td>
<td>0.870</td>
<td>0.306</td>
<td>0.652</td>
<td>–</td>
<td>–0.192</td>
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<tr>
<td>VEP latency</td>
<td>0.043</td>
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<td>0.027</td>
<td>–0.172</td>
<td>–0.179</td>
<td>–0.381</td>
<td>–0.694</td>
<td>–0.215</td>
<td>–</td>
</tr>
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</table>

The difference was obtained as a value of the parameter in the exploration at 2 years minus its value in the baseline exploration. In bold, the correlations which exhibits statistically significant details (p<0.05) with Pearson’s test.

VA: visual acuity; MD: mean deviation; GDx: Laser polarimetry; NFI: nerve fiber index; VEP: visual evoked potentials; OCT: optic coherence tomography; TSNIT: temporal-superior-nasal-inferior.
Longitudinal studies with larger sample sizes and follow-up time would be useful for a deeper understanding of the physiopathology of the disease and to evaluate the usefulness of the RNFL quantitative analysis as a marker of neuronal damage and therapeutic efficiency in clinical trials.

REFERENCES