Clinical and Experimental Ophthalmology 2012; 40: e25–e31 doi: 10.1111/j.1442-9071.2011.02619.x

Original Article

Comparison of retinal nerve fibre layer thickness with visual evoked potential and visual field in patients with multiple sclerosis

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Abstract

Background: To evaluate retinal nerve fibre layer thickness and to compare results with visual evoked potentials and visual field in patients with multiple sclerosis.

Design: A prospective, case-control study, university hospital setting.

Participants: Seventy-three eyes of 37 multiple sclerosis patients and 74 eyes of 37 healthy subjects.

Methods: All patients underwent a complete neurological and ophthalmological examination and peripapillary retinal nerve fibre layer thickness was evaluated using scanning laser polarimetry (GDx). Furthermore, visual evoked potential and visual field testing were performed.

Main Outcome Measures: The χ^2 test, Student's *t*-test, Mann–Whitney *U*-test and Pearson's correlation coefficient analysis of the GDx, visual evoked potential and visual field testing parameters.

Results: GDx measurements showed significantly more retinal nerve fibre layer damage in the patients than in the control groups. Comparison of the GDx parameters between patients with optic neuritis and non-optic neuritis demonstrated a statistically significant difference in symmetry ($P = 0.046$) and superior/nasal parameters ($P = 0.009$). A correlation was found between the number, superior and inferior ratio parameters, and P100 amplitude obtained with visual evoked potential in patients with non-optic neuritis. Additionally, there was a correlation between the number, inferior ratio and superior/nasal parameters, and the mean deviation of visual field in the non-optic neuritis group.

Conclusions: For retinal nerve fibre layer thickness measurements in multiple sclerosis patients, the GDx, along with other techniques, such as visual evoked potential, can be used as a diagnostic and follow-up criterion, particularly in patients without optic neuritis.

Key words: multiple sclerosis, retinal nerve fibre layer, scanning laser polarimetry, standard automated perimetry, visual evoked potential.

Introduction

Multiple sclerosis (MS) is a chronic, demyelinating and idiopathic (possibly autoimmune) disease of the central nervous system (CNS), in which the optic nerve is most susceptible.[1,2] Optic neuritis (ON) is the first manifestation in 15–20% of MS patients, with an additional 40% suffering an attack at some point during the course of the disease. About 75% of patients recover 6/6 vision, but despite this, patients

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Received 25 March 2011; accepted 27 May 2011.

Conflict/competing interest: No stated conflict of interest.

Funding sources: No specific funding.

frequently complain of subjective visual failure.[3–5] Visual evoked potentials (VEPs) have been used as an objective estimation of optic nerve function after recovery from demyelinating ON. Abnormal results have been established in 65–100% of cases with 6/6 vision or better.[4,6,7] The accurate aetiology of these persistent abnormalities is unknown, but studies using fundoscopic examination, direct axonal counting in post-mortem tissue, and optical imaging devices have found that after experiencing an episode of ON, the retinal nerve fibre layer (RNFL) decreases in thickness because of axonal loss. Even in the absence of a history of acute ON, eyes of patients with MS have reduced numbers of retinal ganglion cell axons in pathological studies.[8–12] The mechanism of axonal loss is unknown, but may be due to the retrograde atrophy of axons within plaques of demyelination.[6] Kerrison *et al.*[13] showed RNFL atrophy at post-mortem in 35 of 49 eyes of patients previously diagnosed with MS, and these changes have been correlated with disease activity and white matter lesion volume in neuroradiological studies, such as magnetic resonance imaging.[13–16]

Scanning laser polarimetry (GDx) is a non-invasive, quantitative technique that measures peripapillary retinal nerve fibre layer thickness (RNFLT) using polarized light that undergoes a phase shift after passing through the RNFL.[17] The technique has been widely used in the clinic and in research for detecting RNFL loss.[2,18–20] However, a recent experiment in monkey eyes suggested that GDx can detect axonal degeneration before RNFL thinning occurs, because of its ability to evaluate microtubule density changes.[21]

In this study, we measured the RNFLT using GDx in MS patients and compared the results with those in a control group, and investigated the correlation between the values obtained and the parameters of the visual field and VEP.

Methods

The study was approved by the local ethics committee. Informed written consent was obtained from all patients and volunteers before study entry.

In total, 73 eyes in 37 patients diagnosed with MS by standard clinical and neuroimaging criteria,[22] and 74 eyes in 37 healthy controls were included. For GDx, the Nerve Fiber Analyser (Laser Diagnostic Technologies, Inc., San Diego, CA, USA) was used to prospectively measure the RNFLT. SITA Standard central 30-2 threshold visual field testing (Humphrey Field Analyser II-750; Zeiss Meditec Inc., Dublin, CA, USA) was performed using standard static automated white-on-white perimetry. VEPs were recorded using a Medelec Synergy Multimedia EMG/EP sensor (Oxford Instruments, Abingdon, Oxon, UK).

Of the 37 patients, 27 were women and 10 were men, with ages ranging from 20 to 51 years (mean: 35.2 years). Each patient underwent a complete ophthalmological examination, including measurement of intraocular pressure (Goldmann applanation tonometry), corrected visual acuity, eye motion, pupil reactions, colour vision, and anterior and posterior segment examinations. MS patients who had had attacks of ON within the past 6 months were excluded, to allow adequate time for retrograde degeneration of the RNFL and to minimize the effect of optic disc swelling from acute inflammation.[23–25] Exclusion criteria were as follows: a history of systemic disease, such as systemic hypertension and diabetes mellitus, intraocular pressure >21 mmHg, glaucomatous optic disc changes, spheric and cylindrical refraction greater than +2.0 or −2.0 dioptres, a history of eye surgery and ophthalmic pathology, such as anterior ischemic optic neuropathy, high myopia and congenital abnormalities of the optic nerves, which are likely to have an effect on RNFLT.

The control group consisted of 74 eyes in 37 subjects with ages ranging between 21 and 58 years (mean: 37.9 years); 22 patients were women and 15 were men. In the control group, 34 subjects were emmetropic with complete visual acuity; three had an index of refraction ranging between 0.50 and +1.50 dioptres, with a corrected visual acuity of 1.0, and colour vision testing revealed normal function. The anterior and posterior segment examinations were normal and all patients had intraocular pressure <21 mmHg and cup:disc ratio < 2/10.

Measurement of RNFLT was performed by the same examiner and the mean measurements obtained from three consecutive images with 95% quality were evaluated. After visual field measurements, the two global indices, mean deviation (MD) and pattern standard deviation were also evaluated. The reliability criteria were established as <20% fixation losses, and false-negative and false-positive responses for the visual field results. The N75 (ms), P100 (ms), N145 latencies, and P100 (μV) amplitudes were measured in patients in whom VEP patterning was performed.

The data were analysed using the Statistical Package for the Social Sciences (SPSS) version 13.0 Software for Windows (SPSS Inc., Chicago, IL, USA). The mean values are presented as arithmetic means ± standard error. The χ^2 test, Student's *t*-test and Mann–Whitney *U*-test were used for comparisons between the groups. *P* values <0.05 were considered to indicate statistical significance. Pearson's correlation analysis was used to evaluate correlations between the variables. Correlation coefficients were interpreted as follows: 0–0.250 = weak correlation;

Table 1. The results of nerve fiber layer thickness

Variable	Patient groups ($n = 73$ eyes)	control groups ($n = 74$ eyes)	p
Number	35.5 ± 3.0	15.7 ± 0.7	0.001
Symmetry	0.99 ± 0.02	0.97 ± 0.01	0.507
Superior ratio	2.14 ± 0.06	2.38 ± 0.05	0.002
Inferior ratio	2.23 ± 0.07	2.47 ± 0.05	0.006
Superior/nasal ratios	1.98 ± 0.04	2.16 ± 0.04	0.002
Superior maximum (μm)	78.0 ± 2.3	89.0 ± 2.0	0.001
Inferior maximum (μm)	80.7 ± 2.4	91.4 ± 1.8	0.001
Average thickness (μm)	57.8 ± 1.6	61.7 ± 1.4	0.069
Ellipse average (μm)	61.3 ± 1.6	66.4 ± 1.4	0.015
Superior average (μm)	70.3 ± 1.8	75.8 ± 1.6	0.024
Inferior average (μm)	71.5 ± 2.1	79.3 ± 1.6	0.003

0.251–0.500 = moderate correlation; 0.501–0.750 = strong correlation; and 0.751–1 = very strong correlation. A 95% confidence interval was used to determine statistical significance of study data.

Results

There was no significant difference in age (*t*-test) or gender (χ^2 test) between the two groups ($P = 0.264$, $P = 0.219$, respectively). The number parameter was lower, whereas all other parameters were higher in the patient group compared to the control group. The comparison of the GDx parameters between the patient and control groups revealed statistically significant differences in the number ($P = 0.001$), superior ratio ($P = 0.002$), inferior ratio ($P = 0.006$), superior/nasal ratios ($P = 0.002$), superior maximum ($P = 0.001$), inferior maximum ($P = 0.001$), ellipse average ($P = 0.015$), superior average ($P = 0.024$) and inferior average ($P = 0.003$). There was no statistically significant difference in the symmetry or average thickness parameters (Table 1).

The analysis of correlation between age and disease duration (time from onset of symptoms to GDx evaluation was 55.8 ± 8.6 months) and the GDx parameters revealed a moderate and positive correlation between age and average thickness, ellipse average and superior average parameters ($r = -0.269$, $r = -0.297$, $r = -0.359$) in 37 MS patients. We found a moderate and positive correlation between disease duration and the number parameter ($r = 0.387$), and a moderate and negative correlation between disease duration and superior ratio, inferior ratio, superior/nasal ratios and superior average parameters ($r = -0.408$, $r = -0.314$, $r = -0.385$, $r = -0.306$; Table 2).

Of the 37 MS patients, 16 eyes in 11 patients had a history or finding of ON. This patient group was referred to as the ON (+) group, whereas 58 eyes without a history or finding of ON were referred to as the ON (–) group. The number parameter was found to be higher, whereas all other parameters were lower in the ON (+) group compared with the ON (–) group. GDx parameters were compared between the two groups using the Mann–Whitney *U*-test. A statistically significant difference was found in the symmetry ($P = 0.046$) and superior/nasal ratios parameters ($P = 0.009$). There was no significant difference in the other parameters (Table 3).

In the ON (–) group, the GDx and VEP parameters were statistically compared in 36 eyes in 18 patients in whom GDx and VEP measurement were performed. There was a statistically strong and negative correlation ($r = -0.573$) between the number and P100 (μV) amplitude, and a statistically strong and positive correlation ($r = 0.524$, $r = 0.580$) between the superior ratio and inferior ratio and P100 (μV) amplitude. A statistically moderate and positive correlation ($r = 0.394$, $r = 0.290$, $r = 0.270$) was found between superior/nasal, superior average, inferior average and P100 (μV) amplitude (Table 4).

Table 2. The analysis of correlation between age and duration of the disease and the GDx parameters in patient groups

Variable	Age	Duration of the disease
Number	0.177	0.387*
Symmetry	–0.121	–0.110
Superior ratio	–0.247	–0.408*
Inferior ratio	–0.187	–0.314*
Superior/nasal ratios	–0.130	–0.385*
Superior maximum (μm)	–0.231	–0.172
Inferior maximum (μm)	–0.191	–0.158
Average thickness (μm)	–0.269*	–0.137
Ellipse average (μm)	–0.297*	–0.138
Superior average (μm)	–0.359*	–0.306*
Inferior average (μm)	–0.234	–0.205

GDx: Scanning laser polarimetry.
*indicates moderate correlations between variables.

Table 3. The comparison of the GDx parameters between ON (+) and ON (–) in multiple sclerosis patients

Variable	ON (+)	ON (–)	p
Number	44.3 ± 7.8	33.0 ± 3.0	0.182
Symmetry	0.91 ± 0.03	1.01 ± 0.03	0.046
Superior ratio	1.93 ± 0.12	2.2 ± 0.06	0.060
Inferior ratio	2.18 ± 0.17	2.24 ± 0.07	0.645
Superior/nasal ratios	1.07 ± 0.09	2.05 ± 0.04	0.009
Superior maximum (μm)	70.7 ± 3.7	80.0 ± 2.7	0.215
Inferior maximum (μm)	78.6 ± 4.4	81.2 ± 2.8	0.759
Average thickness (μm)	55.4 ± 2.4	58.5 ± 1.9	0.774
Ellipse average (μm)	58.7 ± 2.5	62.0 ± 1.9	0.815
Superior average (μm)	64.1 ± 3.0	72.0 ± 2.1	0.180
Inferior average (μm)	69.4 ± 3.5	72.1 ± 2.5	0.910

GDx: Scanning laser polarimetry. **ON (+):** The patients with previous history of optic neuritis, **ON (–):** The patients without previous history of optic neuritis.

Table 4. The correlation between GDx and VEP parameters in ON (–) Patients

Variable	N75 ms	P100 ms	N145 μV	P100 μV
Number	0.182	0.175	0.178	–0.573**
Symmetry	0.100	0.039	0.034	–0.088
Superior ratio	–0.074	–0.054	0.043	0.524**
Inferior ratio	–0.140	–0.092	–0.005	0.580**
Superior/nasal ratios	0.116	0.106	0.090	0.394*
Superior maximum (μm)	–0.101	–0.184	–0.199	0.216
Inferior maximum (μm)	–0.107	–0.159	–0.191	0.225
Average thickness (μm)	–0.114	–0.170	–0.165	0.160
Ellipse average (μm)	–0.134	–0.184	–0.184	0.215
Superior average (μm)	–0.136	–0.182	–0.159	0.290*
Inferior average (μm)	–0.102	–0.152	–0.161	0.270*

GDx: Scanning laser polarimetry. **ON (–):** The patients without previous history of optic neuritis. **VEP:** Visual evoked potentials.
*indicates moderate correlations between variables.
**indicates strong correlations between variables.

Table 5. The correlation between GDx and SAP parameters in ON (–) patients

Variable	MD	PSD
Number	–0.296*	0.195
Symmetry	–0.141	0.029
Superior ratio	0.274	–0.120
Inferior ratio	0.316*	–0.146
Superior/nasal ratios	0.284*	–0.242
Superior maximum (μm)	0.006	0.022
Inferior maximum (μm)	0.090	–0.013
Average thickness (μm)	–0.37	0.052
Ellipse average (μm)	–0.29	0.065
Superior average (μm)	0.013	0.028
Inferior average (μm)	0.034	0.035

GDx: Scanning laser polarimetry. **MD:** Mean Deviation. **PSD:** Pattern Standard Deviation. **SAP:** Standard automated perimetry.
*indicates moderate correlations between variables.

In the ON (–) group, GDx and visual field parameters were compared statistically in 35 eyes in 18 patients in whom GDx and visual field measurement were performed. There was a statistically moderate and negative correlation ($r = -0.296$) between the number and MD, and a statistically moderate and positive correlation ($r = 0.316$, $r = 0.284$) between the inferior ratio and superior/nasal and MD (Table 5).

Discussion

In 1972, Hoyt *et al.*[26] first observed the qualitative changes in RNFL in patients with glaucoma and later, a number of other manuscripts have discussed RNFLT using different techniques, such as red-free fundus photography,[27] Heidelberg retinal tomography,[28] GDx[6] or optical coherence tomography,[10] and different types of optic neuropathy.[29–31] However, relatively few studies have described the structural changes in association with functional testing.[7,32,33] In the present study, RNFLT and optic nerve function were assessed using GDx, visual field testing and VEP measurement in MS patients.

In our study, as in a number of previous studies that have investigated RNFLT using different techniques in MS patients,[2,6,7,13,14] RNFLT was significantly decreased in the MS group compared with the control group. The comparison of the GDx parameters between the MS and the control group revealed statistically significant differences in nine of ten parameters: specifically, the number, superior ratio, inferior ratio, superior/nasal ratios, superior maximum, inferior maximum, ellipse average, superior average and inferior average. The comparison of the GDx parameters between MS eyes with ON history (16 eyes, 21.9%) and MS eyes without ON history (57 eyes, 78.1%) showed a significant difference in the symmetry and nasal/superior ratios parameters.

MS is a progressive disease in which subclinical RNFL thinning may occur, even in patients who have not been clinically diagnosed with ON.[8,10,14] A recent study by Quelly *et al.*[2] concluded that 20–40% of MS eyes with no clinical history of ON are likely to have had a subclinical event somewhere along the visual pathway.

In the present study, as in previous studies, RNFLT was decreased in MS eyes without ON history. Although ON is usually unilateral, which may result in axonal loss, it can be present in the opposite eye. The mechanism for this axonal loss is unclear, but it may be that clinically silent demyelinating lesions within the optic nerve, associated with axonal damage with retrograde degeneration in the RNFL have occurred. However, RNFLT measurement can be effective in detecting a history of silent ON for the assessment of subclinical neuropathy in MS patients.

Several studies have extensively investigated VEP results in MS patients. Weinstock-Guttman *et al*.[22] found a lengthening of the P100 latency in 75% of MS patients and reported a strong correlation between these findings and RNFLT. In a study by MacFadyen *et al*.,[7] RNFL defects and neuroretinal rim area were quantitatively detected and compared with VEP in MS patients. They found local or diffuse RNFL defects in 54% of the patients, abnormally small neuroretinal rim area in 30%, and abnormal VEP latencies in 63% of all patients. Pueyo *et al*.[20] also found a strong correlation between VEP and GDx parameters in MS patients. Parisi *et al*.,[10] however, compared eyes in 14 MS patients with a history of ON and those in a control group, and found that RNFL measurements had a correlation with pattern electroretinograms, but no correlation with VEP results. Prospective randomized studies are required to define better the correlation between RNFLT and VEP.

In the present study, the GDx and VEP parameters were compared statistically in 36 eyes in 18 patients in the group without optic neuropathy. Of the GDx parameters, there was a strong correlation between the number, superior ratio and inferior ratio, and P100 (μV) amplitude. We also found a moderate correlation between superior/nasal ratios, superior average, inferior average and P100 (μV). Based on these findings, we conclude that co-evaluation of GDx and VEP parameters has diagnostic value in patients with subclinical ON who have no history or finding of ON.

Several studies have demonstrated a correlation between RNFLT and visual field in MS patients.[32–35] Although the authors agree on this correlation in ON (+) eyes, different views have been reported for ON (–) eyes. Kitsos *et al*.[1] have reported that a decrease in RNFLT was correlated with the visual field in MS ON (+) and MS ON (–) patients, whereas Cheng *et al*.[34] found this correlation in ON (+) but not ON (–) eyes. Pueyo *et al*.[20] found that about one-third of MS ON (–) eyes, with normal visual acuity and visual fields, showed RNFL defects on optical coherence tomography (30%) or GDx (32.5%) measurements.

Although the parameters that are considered to be most reliable and sensitive for the differentiation of glaucomatous from healthy eyes have varied among studies, the measurement of RNFLT in the superior and inferior quadrants has been reported to be correlated with visual field defects.[30,32] Additionally, Kwon *et al*.[33] reported that the number and ellipse modulation are the two parameters that have the strongest correlation with visual field loss. Furuichi *et al*.[35] reported that there was a significant correlation between RNFLT and MD, whereas Cheng *et al*.[34] suggested that all sectors of the visual field were similarly affected.

In the present study, we found a moderate correlation between the MD of the visual field and the number, inferior ratio and superior/nasal ratios. Patients with unaffected visual acuity and visual function may have a normal visual field. As demonstrated in our study, in these patients, RNFL defects may be present to an extent that does not result in visual field loss. Furthermore, although eyes without optic neuropathy may have normal visual acuity, there may be visual dysfunction, which indicates that RNFL defects and visual field anomalies are present in eyes without optic neuropathy. In the present study, however, the correlations obtained were not very high and there was no correlation with the other parameters, which can be attributed to the fact that visual field testing could not be carried out properly in MS patients because of poor cooperation and general condition.

Some studies have found that patients with a longer history of MS have a more severe reduction in RNFLT,[8,9,36] but others have not.[2,3,37] Henderson *et al*.[37] found no significant correlation between disease duration and RNFLT, but suggested that thinning occurs predominantly in the temporal quadrant. In a study of 299 MS patients with 18 months of follow-up, Talman *et al*.[36] found that RNFL thinning occurred in some patients with MS, even in the absence of a previous history of ON.

In our study, there was a moderate and positive correlation ($r = 0.387$) between disease duration and the number, whereas there was a moderate and negative correlation between disease duration and superior ratio, inferior ratio, superior/nasal ratios and superior average ($r = -0.408$, $r = -0.314$, $r = -0.385$, $r = -0.306$, respectively). Although the correlation between disease duration and RNFLT is controversial, there appears to be a higher probability of correlation between disease duration and RNFLT. Future longitudinal studies, rather than cross-sectional analyses, should be conducted to study RNFLT changes over time.

GDx is relatively reliable, easy to use and quick in the assessment of RNFL compared with other techniques. GDx can detect the decrease in RNFLT in eyes with a previous attack of ON. Thus, in the assessment of RNFL, GDx is a useful alternative diagnostic tool for the identification of patients with a previous history of ON or subgroups who exhibit no fundus findings, such as retrobulbar neuritis, and for the detection of subclinical attacks in a MS patient with no previous attack of ON or visual complaints.

Acknowledgement

We express our sincere gratitude for support and valuable contributions to Dr Sebnem Hanioglu Kargi

from Department of Ophthalmology, the School of Medicine, Zonguldak Karaelmas University, Zonguldak, Turkey. This article is dedicated to Dr Sebnem Hanioglu Kargi, who passed away before the completion of this paper.

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