Optical Coherence Tomography: a window to multiple sclerosis?

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Resumo

Introdução:

A esclerose múltipla (EM) é a doença inflamatória desmielinizante imunomediada que mais frequentemente afecta o sistema nervoso central. Aproximadamente 20% dos doentes têm nevrite óptica como manifestação inicial da doença. A sua frequência, heterogeneidade e ausência de tratamento curativo tornam esta patologia um desafio diagnóstico e terapêutico tanto para o doente como para o médico.

Material e métodos:

Estudo retrospectivo de 133 olhos de 87 indivíduos, 38 doentes com esclerose múltipla (Grupo 1), 9 doentes com nevrite óptica isolada (Grupo 2) e 40 indivíduos saudáveis (Grupo 3) da Consulta de Neuroftalmologia do Centro Hospitalar de Lisboa Central, através da consulta do processo e exames complementares de diagnóstico, entre Janeiro de 2013 e Agosto de 2015.

Foram caracterizados os doentes de acordo com o género, idade, anos de seguimento, melhor acuidade visual corrigida, presença de outras anomalias oculares concomitantes e episódios de nevrite óptica. Foram excluídos doentes com outras patologias oculares.

Foi realizada uma tomografia de coerência óptica (OCT *Spectralis*, *Heidelberg Engineering*) da região macular, seguida de segmentação automática das camadas retinianas.

O estudo estatístico permitiu calcular diferenças estatisticamente significativas entre os diferentes grupos do estudo.

Resultados:

Nos 87 doentes avaliados, a média de idades do grupo 1 foi de 41.55 anos, versus 36.88 do grupo 2 e 44.73 do grupo de controlo. A média da idade do diagnóstico nos doentes com esclerose múltipla foi de 33.73 anos e do tempo de seguimento de 8.01 anos e no grupo da nevrite óptica isolada de 34.33 anos, com 2.88 anos de seguimento. A melhor acuidade visual corrigida variou entre 0.2 e 1.0 no grupo 1 e 0.5 a 1.0 no grupo 2.

Nos doentes com antecedentes de nevrite óptica isolada ou associada a esclerose múltipla observou-se uma diminuição significativa ($p<0.05$) da espessura média da retina assim como diminuição da camada de fibras nervosas, da camada de células ganglionares e da plexiforme interna em relação ao grupo controlo.

Conclusões:

As espessuras, especialmente das camadas mais internas da retina (camada de fibras nervosas, camada de células ganglionares e plexiforme interna), estavam reduzidas de forma estatisticamente significativa ($p<0.05$) em relação ao grupo de controlo.

Palavras-Chave: Esclerose Múltipla; Tomografia de Coerência Óptica; Nevrite Óptica; Camada de células ganglionares

Abstract

Introduction:

Multiple sclerosis (MS) is an inflammatory immune-mediated demyelinating disease that frequently affects the central nervous system. Approximately 20% of patients have optic neuritis has the first manifestation of the disease. Its frequency, heterogeneity and absence of definitive treatment make it a therapeutic and diagnostic challenge for both the patient and the clinician.

Material and methods:

Retrospective study of 133 eyes of 87 patients, 38 patients with MS (Group 1), 9 patients with isolated optic neuritis (Group 2) and 40 healthy patients (Group 3) from the Neurophthalmology Department of Centro Hospitalar de Lisboa Central from January 2013 to August 2015.

Patients were characterized according to gender, age, years of follow-up, best corrected visual acuity, presence of other ocular disorders and episodes of optic neuritis. Patients with other ocular pathologies were excluded.

Optical coherence tomography (OCT Spectralis, Heidelberg Engineering) of the macular region was done in all patients, followed by automatic segmentation of the retinal layer. Statistical tests were made to calculate statistically significant results between different groups.

Results:

Of the 87 patients evaluated, the mean age in Group 1 was 41.55 compared to 36.88 in Group 2 and 44.73 in the control group. Mean age of diagnosis for patients with MS was 33.73 years with a mean follow up of 8.01 years, whereas patients with isolated optic neuritis had a mean age of diagnosis of 34.33 years, with a mean follow-up of 2.88 years. Best corrected visual acuity ranged from 0.2 to 1.0 in Group 1 and 0.5 to 1.0 in Group 2.

In patients with previous episodes of optic neuritis isolated or associated with multiple sclerosis we observed a statistically significant ($p<0.05$) reduction of the average retinal thickness as well as a thinner retinal nerve fiber layer, ganglion cell layer and inner plexiform layer when compared to the control group.

Conclusion:

Thickness of the internal retinal layers was reduced (retinal nerve, ganglion cell and inner plexiform layer) in a statistically significant value ($p<0.05$) when compared to the control group.

Keywords: Multiple sclerosis; optical coherence tomography; optic neuritis; ganglion cell layer

Introduction

Multiple sclerosis (MS) is a chronic, predominantly immune-mediated, inflammatory and demyelinating disease of the central nervous system (CNS) characterized by subacute neurologic deficits correlating with CNS lesions, separated in time and space, excluding other possible diseases. Approximately 20% of MS patients present with acute optic neuritis (AON) as a first demyelinating event and about 40 to 70% experience ON at some point during the course of the disease.[1]

Optical coherence tomography (OCT) is a noninvasive, noncontact, reproducible, quantitative technique that uses interference patterns produced by low coherence light to generate high-resolution images (<5μm) from optical scattering media (e.g. retina). This technique has been used to define and differentiate specific forms of optic neuropathy, document changes in retinal nerve fiber layer thickness (RNFL) and macular volume[2] over time and help correlate structural retinal nerve fiber layer changes with visual function.

Since the RNFL is composed only of unmyelinated axons of the retinal ganglion cells (which convey information from the retina to the lateral geniculate nucleus), measuring RNFL thickness can be a viable method for monitoring axonal loss in MS patients. Following the publication of the first quantitative OCT study of RNFL thickness in multiple sclerosis (MS) in 1999,[1,3] there has been a rapidly growing interest in various applications of OCT in the diagnosis and management of patients with MS, both in those with optic neuritis (MS ON+) and those without optic neuritis (MS ON-).

Subsequently, OCT studies turned to the segmentation of retinal layers from which the individual retinal layers can be delimited, qualitatively and quantitatively assessed to extend the analysis of axonal damage (RNFL) to neuronal degeneration. Ganglion cell layer has been proved to be a sensitive marker to detect damage even in the absence of a previous episode of ON.[3]

This study aims to determine changes in thickness of eight different retinal layers in patients with MS ON+, MS ON- and clinically isolated syndrome (demyelinating lesion suggestive of MS that does not fulfill the criteria for MS diagnosis) and compare them with healthy subjects by using an automated segmentation program of spectral domain optical coherence tomography.

Since the heterogeneity of the MS disease course remains a challenge for patient management and treatment, our study tries to evaluate if inner retinal thickness can serve as a direct biomarker for monitoring the course of this disease.

Material and methods

Retrospective study of 133 eyes of 87 individuals, 47 patients and 40 healthy controls from the Multiple Sclerosis and Neuro-Ophthalmology Department of Hospital de Santo António dos Capuchos, between January 2013 and August 2015.

The design of this study followed the Declaration of Helsinki principles. All participants provided written informed consent.

Patients were characterized according to gender, age, years of follow-up, episodes of optic neuritis and treatment. All participants underwent a full ophthalmologic examination including best corrected visual acuity, biomicroscopy, Goldmann applanation tonometry, and ophthalmoscopy. Participants were excluded if there was any history of a comorbidity that could plausibly account for the presence of macular or neuropathy, including a prior history of glaucoma, uveitis, diabetes, retinal vein occlusion, age-related macular degeneration, retinosquisis, or refractive errors of more or less than six diopters. This happened in two patients with subclinical epiretinal membrane and macular edema.

Study participants were recruited and divided in three groups: Group 1 included 38 patients with MS, subdivided in eyes with optic neuritis (ON+) (n=44), or without optic neuritis (ON-)(n= 31); Group 2 with 9 patients with clinically isolated syndrome (CIS) subdivided in eyes with optic neuritis (ON+) (n=10) or without optic neuritis (ON-) (n=8); Group 3 of healthy controls (n=40).

Patients with MS were diagnosed according to the 2010 modified McDonald criteria by treating neurologists. In patients with CIS, other diseases such as neuromyelitis optica (NMO), and chronic relapsing inflammatory neuropathy were excluded (negative NMO-IgG). All were treated with corticosteroids and one had another episode of optic neuritis in the other eye during follow-up. Disease duration was defined as the time from the first clinical symptom attributable to multiple sclerosis to the date of OCT evaluation. Patients with acute optic neuritis or optic disc edema within three months of the examination were excluded from the study.

All patients underwent retinal OCT imaging and automated retinal segmentation to identify each retinal layer and to quantify its thickness. OCT was performed by an experienced operator using spectral-domain OCT (*Heidelberg Spectralis, Heidelberg Engineering, Germany*).

Macular scanning was obtained and qualitatively assessed for the presence of other macular pathologies. Macular segmentation was performed using Heidelberg Eye Explorer software provided by the manufacturer. Corresponding data was obtained from single horizontal axial scans to separate the retina into 8 layers: 1- nerve fiber layer; 2- ganglion cell layer; 3- inner plexiform layer; 4- inner nuclear layer; 5- outer plexiform layer; 6- outer nuclear layer; 7- photoreceptor layer; 8- retinal pigment epithelium.

Scans were centered at the fovea and obtained at a radius of 3 to 6 mm from the central, superior, inferior, nasal and temporal sectors. Values of mean thickness, nasal (N), superonasal

(SN), inferonasal (IN), temporal (T), temporo-nasal (TN) and superonasal (SN) thickness were collected from all retinal layers. All scans were assessed before segmentation analysis and low quality scans were rejected.

Statistical analysis was performed using SPSS software. Mean layer and sectorial thickness were compared between the different groups using Student t test, considering P values less than 0.05 statistically significant.

Results

In this retrospective study of 133 eyes of 87 individuals, 47 patients and 40 healthy controls, participants were recruited and divided in three groups (Table I):

- Group 1 included 75 eyes of 38 patients with MS, subdivided in eyes with previous acute optic neuritis (ON+, N=44) or without previous optic neuritis (ON-, N= 31). In this group mean age was 41.55 ± 12.38 years, with a mean follow-up of 8.02 ± 8.24 years.
- Group 2 included 18 eyes of 10 patients with clinically isolated syndrome (CIS) subdivided in eyes with optic neuritis (ON+, N=10) or without optic neuritis (ON-, N=8). These patients had a mean age of 34.89 ± 11.75 years and a mean follow-up of 2.88 ± 3.09 years.
- Group 3 (control) included 40 eyes of 40 healthy controls with a mean age of 45.62 ± 13.70 years.

Table I. Baseline demographic and clinical characteristics.

	Group 1 (MS patients)	**Group 2** (CIS patients)
Patients (eyes)	38 (75)	9 (18)
Age, years, mean (SD)	41.55 (±12.38)	34.89 (±11.75)
Female (%)	25 (65.79%)	7 (77.78%))
BCVA (Snellen)	0.862 (±0.21)	0.986 (±0.05)
Disease duration, years	8.01 (±8.24)	2.88 (±3.09)
Eyes with a previous history of AON	44 (58.7%)	10 (55%)

Patient age ranged from 21 years to 68 years (Group 1 and 2) and the ratio of women to men was approximately 2.13:1 (32 women, 15 men).

No significant difference in age was found between groups (two-sample student's t test, $p>0.05$) but Group 1 and 2 were found to differ significantly regarding disease duration (two-sample student's t test, $p<0.001$).

All 87 patients underwent retinal OCT imaging and automated retinal segmentation to identify each retinal layer and to quantify its thickness.

Average total retinal thickness

In this study the average total retinal thickness was initially compared between MS ON + eyes and MS ON- of Group 1 and 2 with the eyes of control Group 3. This revealed a statistical significant difference (Student T test, $p< 0.05$) between MS ON+ and the control group, demonstrating a reduced macular thickness in MS ON + eyes at the fovea and also all sectors (Table II). Although MS ON- eyes didn't reveal a statistically significant reduction at the fovea, they did in all other sectors except in T6 (Table III).

There was no statistical difference in macular thickness between MS ON+ and ON- eyes.

Both CIS ON+ and CIS ON- eyes did not reveal a statistical significant difference (Student T test, $p<0.05$) between MS ON+ and the control group, demonstrating a reduced macular thickness in MS ON + eyes at the fovea and also at all sectors.

Table II. Mean thickness of all 10 retinal layers (central and sectorial) obtained by automatic segmentation of the Spectralis OCT in MS ON+ eyes and control group.

Inner ring	Outer Ring	MS ON+ (N=31)		Control Group (N=40)		p Value	
Central total retinal thickness, µm, mean		268.00		277.68		**0.04**	
Superior	Superior	324.05	287.90	346.80	301.55	**0.001**	**0.001**
Temporal	Temporal	312.52	280.87	331.18	285.85	**0.001**	**0.001**
Inferior	Inferior	318.45	282.39	340.50	291.40	**0.001**	**0.001**
Nasal	Nasal	331.65	298.19	347.40	316.68	**0.001**	**0.001**
Central RNFL thickness, µm, mean		13.26		12.65		0.713	
Superior	Superior	25.52	34.84	24.68	37.40	**0.033**	**0.001**
Temporal	Temporal	19.35	21.68	17.33	18.80	0.118	0.169
Inferior	Inferior	23.55	36.26	25.00	38.98	**0.005**	**0.001**
Nasal	Nasal	21.58	42.06	20.88	48.33	0.458	**0.001**
Central GCL thickness, µm, mean		14.84		15.70		**0.008**	
Superior	Superior	44.61	31.16	51.58	35.55	**0.001**	**0.001**
Temporal	Temporal	40.03	31.74	46.80	35.90	**0.001**	**0.001**
Inferior	Inferior	42.87	31.68	51.35	34.63	**0.001**	**0.001**
Nasal	Nasal	41.55	34.00	51.38	39.00	**0.001**	**0.001**
Central IPL thickness, µm, mean		21.26		21.77		**0.001**	
Superior	Superior	37.29	25.65	41.77	28.59	**0.001**	**0.001**
Temporal	Temporal	38.61	30.06	41.56	32.36	**0.001**	**0.001**
Inferior	Inferior	37.52	25.48	40.26	27.97	**0.001**	**0.001**
Nasal	Nasal	37.90	26.03	42.67	29.51	**0.001**	**0.001**
Central INL thickness, µm, mean		19.48		20.15		0.199	
Superior	Superior	41.55	30.90	41.00	32.51	0.773	**0.011**
Temporal	Temporal	37.61	33.35	36.87	33.67	0.364	0.714
Inferior	Inferior	40.39	31.13	39.74	31.72	0.443	0.864
Nasal	Nasal	41.10	34.10	39.79	34.64	0.435	0.656
Central OPL thickness. µm, mean		26.19		25.87		0.484	
Superior	Superior	32.42	26.32	34.62	27.05	0.443	0.433
Temporal	Temporal	32.39	27.29	30.97	26.79	0.865	0.401
Inferior	Inferior	33.71	26.71	33.33	26.74	0.647	0.521
Nasal	Nasal	32.58	28.32	34.05	28.72	0.898	0.987
Central ONL thickness, µm, mean		91.26		93.79		0.183	
Superior	Superior	70.58	60.74	69.21	61.00	0.835	0.319
Temporal	Temporal	73.55	56.68	74.41	58.62	0.695	0.222
Inferior	Inferior	70.06	54.19	68.08	53.46	0.928	0.377
Nasal	Nasal	74.87	56.77	74.46	57.62	0.614	0.224
Central RPE, µm, mean		17.10		17.10		0.139	
Superior	Superior	15.26	14.39	15.28	13.21	0.439	0.099
Temporal	Temporal	14.48	12.84	14.33	12.74	0.173	0.269
Inferior	Inferior	14.97	13.06	14.64	13.00	0.585	0.673
Nasal	Nasal	15.10	13.48	15.49	13.31	0.506	0.425
Central FR thickness. µm, mean		89.87		89.33		0.255	
Superior	Superior	80.81	78.48	81.36	78.85	0.970	0.455
Temporal	Temporal	81.23	77.55	81.54	78.05	0.757	0.886
Inferior	Inferior	80.29	76.52	80.18	77.51	0.539	0.146
Nasal	Nasal	82.19	78.26	82.51	78.77	0.425	0.930

Table III. Mean thickness of all 10 retinal layers (central and sectorial) obtained by automatic segmentation of the Spectralis OCT in MS ON- eyes and control group.

Inner ring	Outer Ring	MS ON- (n=31)		Control Group (n=40)		p Value	
Central total retinal thickness, µm, mean		274.35		277.68		0.515	
Superior	Superior	332.03	287.90	346.80	301.55	**0.001**	**0.001**
Temporal	Temporal	318.06	280.87	331.18	285.85	**0.028**	0.310
Inferior	Inferior	328.94	282.39	340.50	291.40	**0.021**	**0.053**
Nasal	Nasal	331.65	298.19	347.40	316.68	**0.002**	**0.001**
Central RNFL thickness, µm, mean		13.26		12.65		0.323	
Superior	Superior	25.52	34.84	24.68	37.40	0.485	0.086
Temporal	Temporal	19.35	21.68	17.33	18.80	**0.048**	**0.042**
Inferior	Inferior	23.55	36.26	25.00	38.98	0.220	0.138
Nasal	Nasal	21.58	42.06	20.88	48.33	0.534	**0.010**
Central GCL thickness, µm, mean		14.84		15.70		0.439	
Superior	Superior	44.61	31.16	51.58	35.55	**0.005**	**0.001**
Temporal	Temporal	40.03	31.74	46.80	35.90	**0.004**	**0.003**
Inferior	Inferior	42.87	31.68	51.35	34.63	**0.001**	**0.006**
Nasal	Nasal	41.55	34.00	51.38	39.00	**0.001**	**0.001**
Central IPL thickness, µm, mean		21.26		21.77		0.552	
Superior	Superior	37.29	25.65	41.77	28.59	**0.001**	**0.001**
Temporal	Temporal	38.61	30.06	41.56	32.36	**0.014**	**0.007**
Inferior	Inferior	37.52	25.48	40.26	27.97	0.107	**0.004**
Nasal	Nasal	37.90	26.03	42.67	29.51	**0.002**	**0.001**
Central INL thickness, µm, mean		19.48		20.15		0.618	
Superior	Superior	41.55	30.90	41.00	32.51	0.538	**0.010**
Temporal	Temporal	37.61	33.35	36.87	33.67	0.372	0.664
Inferior	Inferior	40.39	31.13	39.74	31.72	0.519	0.439
Nasal	Nasal	41.10	34.10	39.79	34.64	0.174	0.432
Central OPL thickness. µm, mean		26.19		25.87		0.818	
Superior	Superior	32.42	26.32	34.62	27.05	0.249	0.270
Temporal	Temporal	32.39	27.29	30.97	26.79	0.341	0.487
Inferior	Inferior	33.71	26.71	33.33	26.74	0.851	0.967
Nasal	Nasal	32.58	28.32	34.05	28.72	0.442	0.659
Central ONL thickness, µm, mean		91.26		93.79		0.341	
Superior	Superior	70.58	60.74	69.21	61.00	0.615	0.888
Temporal	Temporal	73.55	56.68	74.41	58.62	0.744	0.399
Inferior	Inferior	70.06	54.19	68.08	53.46	0.491	0.698
Nasal	Nasal	74.87	56.77	74.46	57.62	0.878	0.643
Central RPE, µm, mean		17.10		17.10		0.993	
Superior	Superior	15.26	14.39	15.28	13.21	0.959	0.063
Temporal	Temporal	14.48	12.84	14.33	12.74	0.738	0.723
Inferior	Inferior	14.97	13.06	14.64	13.00	0.431	0.820
Nasal	Nasal	15.10	13.48	15.49	13.31	0.355	0.633
Central FR thickness. µm, mean		89.87		89.33		0.713	
Superior	Superior	80.81	78.48	81.36	78.85	0.506	0.537
Temporal	Temporal	81.23	77.55	81.54	78.05	0.712	0.354
Inferior	Inferior	80.29	76.52	80.18	77.51	0.859	0.064
Nasal	Nasal	82.19	78.26	82.51	78.77	0.692	0.362

Retinal Layer Thickness

After comparing total macular thickness, the thickness of all eight retinal layers was measured (RNFL, GCL, IPL, INL, OPL, ONL, RPE, FR) with scans centered at the fovea and at a radius of 3 to 6 mm from the central, superior, inferior, nasal and temporal sectors. Values of mean thickness, nasal (N), superonasal (SN), inferonasal (IN), temporal (T), temporo-nasal (TN) and superonasal (SN) thickness were collected from all retinal layers (Table IV).

Retinal Nerve Fiber Layer:

Statistical significant differences ($p<0.05$) were found between MS ON+ eyes and Control Group 3, with a reduction in average retinal thickness at the RNFL, with a more significant reduction in respectively the superior and inferior sectors. Although a statistical significance was not obtained in the temporal and nasal sectors, it showed a statistical trend towards reduction. This reduction did not respect the ISNT rule for glaucomatous eyes. MS ON- only showed a significant reduction in the temporal sector of both inner and outer ring and in the nasal sector of the outer ring. Superior RNFL thickness was reduced in MS ON+ compared to MS ON- ($p<0.05$). CIS ON- had a significantly reduced RNFL thickness ($p<0.05$) in the superior sector of the inner and outer ring whereas CIS ON+ had a temporal and nasal reduction also of both rings.

Ganglion Cell Layer:

GCL was found to be significantly thinner ($p<0.05$) in both MS ON+ and MS ON- eyes compared with Control Group 3, with a reduction in average retinal thickness in all sectors except for the central one. Central GCL thickness was reduced in MS ON+ compared to MS ON- ($p<0.05$). CIS ON- had a significant reduced GCL thickness ($p<0.05$) in the superior sector of the outer ring whereas CIS ON- did not show a significant reduction.

Inner Plexiform Layer:

The calculated measurements revealed a thinner IPL in all sectors ($p<0.001$) in MS ON+ and in all sectors except for the central one in MS ON- compared to the control group. IPL was significantly reduced in MS ON+ in contrast with MS ON- in all sectors except for the nasal outer ring. IPL was significantly thinner in CIS ON+ eyes compared to the control group only in the superior outer ring. CIS ON- did not appear to have a significant reduction.

Inner Nuclear Layer, Outer Plexiform Layer, Outer Nuclear Layer, Retinal Pigment Epithelium and Photoreceptor Layer:

Both MS ON+ and MS ON- patients did not demonstrate statistically significant differences when compared to the Control Group. A trend was observed with an increase in thickness in some sectors of the INL-OPL and ONL-RPE layer although this was not statistically significant. CIS ON+ and ON- eyes exhibited the same trend.

Table IV. Mean thickness of all 10 retinal layers (central and sectorial) obtained by automatic segmentation of the Spectralis OCT in MS ON+ eyes and MS ON-.

Inner ring	Outer Ring	MS ON+ (N=44)		MS ON- (N=31)		p Value	
Central total retinal thickness, µm, mean		268.00		274.35		0.200	
Superior	Superior	324.05	281.20	332.03	287.90	0.151	0.143
Temporal	Temporal	312.52	271.34	318.06	280.87	0.404	0.067
Inferior	Inferior	318.45	273.61	328.94	282.39	0.077	0.109
Nasal	Nasal	324.39	291.61	331.65	298.19	0.205	0.329
Central RNFL thickness, Mm, mean		12.43		13.26		0.259	
Superior	Superior	22.68	30.86	25.52	34.84	**0.038**	**0.039**
Temporal	Temporal	18.16	20.45	19.35	21.68	0.249	0.512
Inferior	Inferior	22.36	32.73	23.55	36.26	0.374	0.148
Nasal	Nasal	20.34	37.91	21.58	42.06	0.319	0.189
Central GCL thickness, µm, mean		12.98		14.84		**0.034**	
Superior	Superior	41.52	29.89	44.61	31.16	0.198	0.200
Temporal	Temporal	36.07	29.50	40.03	31.74	0.159	0.152
Inferior	Inferior	40.09	29.50	42.87	31.68	0.307	0.062
Nasal	Nasal	39.09	31.50	41.55	34.00	0.393	0.076
Central IPL thickness, µm, mean		19.20		21.26		**0.007**	
Superior	Superior	33.77	24.36	37.29	25.65	**0.019**	**0.113**
Temporal	Temporal	33.80	27.82	38.61	30.06	**0.002**	**0.021**
Inferior	Inferior	33.39	24.16	37.52	25.48	**0.012**	**0.128**
Nasal	Nasal	34.43	24.36	37.90	26.03	**0.049**	0.107
Central INL thickness, µm, mean		18.50		19.48		0.337	
Superior	Superior	41.23	31.07	41.55	30.90	0.721	0.769
Temporal	Temporal	37.52	33.43	37.61	33.35	0.911	0.915
Inferior	Inferior	40.36	31.84	40.39	31.13	0.979	0.337
Nasal	Nasal	40.43	34.39	41.10	34.10	0.401	0.672
Central OPL thickness, µm, mean		24.91		26.19		0.369	
Superior	Superior	33.32	26.57	32.42	26.32	0.473	0.646
Temporal	Temporal	30.77	27.25	32.39	27.29	0.217	0.952
Inferior	Inferior	34.11	27.18	33.71	26.71	0.788	0.481
Nasal	Nasal	34.30	28.70	32.58	28.32	0.361	0.622
Central ONL thickness, µm, mean		90.30		91.26		0.717	
Superior	Superior	69.70	59.30	70.58	60.74	0.730	0.452
Temporal	Temporal	73.52	56.68	73.55	56.68	0.991	0.998
Inferior	Inferior	68.32	51.89	70.06	54.19	0.498	0.205
Nasal	Nasal	73.02	55.36	74.87	56.77	0.558	0.518
Central RPE, µm, mean		18.20		17.10		0.150	
Superior	Superior	15.64	13.93	15.26	14.39	0.354	0.367
Temporal	Temporal	14.95	13.05	14.48	12.84	0.244	0.471
Inferior	Inferior	14.86	13.11	14.97	13.06	0.815	0.857
Nasal	Nasal	15.80	13.59	15.10	13.48	0.135	0.758
Central FR thickness, µm, mean		90.75		89.87		0.483	
Superior	Superior	81.39	79.23	80.81	78.48	0.434	0.176
Temporal	Temporal	81.77	77.98	81.23	77.55	0.456	0.439
Inferior	Inferior	80.57	76.80	80.29	76.52	0.690	0.597
Nasal	Nasal	83.14	78.82	82.19	78.26	0.264	0.337

Clinically isolated syndrome

Eyes with CIS ON+ and CIS ON- did not show statistically significant differences between them in any retinal layer except for a reduction in the RPE central layer in CIS ON- ($P<0.02$).

Discussion

Multiple sclerosis is a central nervous system disease characterized by inflammation and neuro-axonal degeneration. The eye itself is commonly affected by MS. Optic neuritis is a frequent inaugural sign of MS ant it can also develop during the course of this disease. During acute ON, fluorescein leakage has proved that a disruption of the blood-retina barrier occurs as well as showed retinal perivascular inflammation (periphlebitis). Studies demonstrated that active periphlebitis could be a risk factor for relapses and brain lesions.[4] Intermediate uveitis (pars planitis) can also occur in up to 15-25% of patients.[5]

Neuro-axonal degeneration affecting the retina can be structurally and functionally related to pathology of the visual pathways. Inflammation and activation of the microglia have also been demonstrated in the inner retina of MS patients but the relationship between inflammation and neurodegeneration needs more studies.

Optical Coherence Tomography (OCT) has become one of the most important tools in ophthalmology practice and especially in neuro-ophthalmology practice. It is a fast, reliable and non-invasive method that can provide high quality images of the optic nerve, retinal nerve fiber layer, macular volume and ganglion cell layer which can be relevant for diagnosis, prognosis and follow-up of different diseases.

In MS, OCT can have a potential role in quantifying axonal loss and assessing longitudinal alterations.[6] The first studies with time-domain OCT showed that peripapillary RNFL thinning was associated with macular volume reduction in eyes with previous MS ON+.[1] More recently, spectral-domain OCT studies confirm that eyes with thinner macula were associated with reduced thickness of the macular RNFL, ganglion cell layer (GCL), and inner plexiform layer (IPL).[3,7]

The temporal and spatial associations between axonal injury and ganglion cell loss have yet to be determined, although retrograde degeneration of the RNFL has been implicated as the most important mechanism leading to macular damage. It seems that in a subset of patients with MS, a disproportionate thinning of the macular inner and outer nuclear layers has been reported in the presence of normal peripapillary RNFL thickness and these patients may present a MS-related macular pathology as a primary process independent of optic nerve pathology.[8]

Recently various protocols of segmentation of OCT images, both manual and automated [7,9,10], have been used to quantify changes in different retinal layers in patients with MS, with variable levels of success.[11] These segmentation algorithms rely on techniques such as edge detection, intensity variations, active contours, multiresolution hierarchical support vector

machines and graph-cut segmentation. Most of these approaches introduce complex models or methodologies to obtain reliable results and are therefore very computationally intensive. Previous results of manual and automatic segmentation have yielded similar results insofar, except for the RNFL and INL layers. These layers are thinner than others making it difficult to determine their limits correctly. One study suggests that segmentation of both layers together with their neighboring layers, namely RNFL-GCL-IPL together, and similarly the INL-OPL, can be a method to overcome this issue. This approach is also applied by other widely used automatic segmentation methods. However, Spectralis OCT is effective in distinguishing and measuring these layers when compared to the Cirrus OCT used in the first studies. [11]

In our study, we introduce a fast approach for automatic segmentation of 8 retinal layers: 1- retinal nerve fiber layer (RNFL); 2- ganglion cell layer (GCL); 3- inner plexiform layer (IPL); 4- inner nuclear layer (INL); 5- outer plexiform layer (OPL); 6- outer nuclear layer (ONL); 7- photoreceptor layer (FR); 8- retinal pigment epithelium (RPE). To validate our method in a real-world setting, we quantified retinal changes caused by ON in two separate cohorts of patients with MS and CIS, comparing them with a control group of healthy aged-matched individuals.

Our results revealed a significant reduction in average macular layer and retinal nerve fiber layer in MS patients with previous episodes of optic neuritis and even in the absence of optic neuritis, when compared to the control group. However, there was no statistical significance in this reduction between MS ON+ and MS ON- except for a greater reduction in the superior sector in MS ON+ eyes. This finding is in line with previous studies that show a reduce thickness of both the macula and RNFL in MS patients. [7,9,10,11,12]

In our study, GCL and IPL layers were also thinned in both MS ON+ and MS ON- with this thickness being more pronounced in eyes with previous optic neuritis. Thinning in ON- eyes can be explained perhaps by subclinical episodes of optic neuritis, degeneration of the ganglion cells and their axons without inflammation or retrograde degeneration of MS lesions. This type of trans-synaptic degeneration has been reported in patients with homonymous hemianopia due to retrogeniculate lesions. *Albrecht et al* [13] applied manual segmentation to a similar cohort of patients obtaining the same results.

We did not detect alterations in other layers except for a trend in the increase in thickness in some sectors of the INL-OPL and ONL-RPE layer although this was not statistically significant. This can be because the ON episodes did not affect outer retinal layers or retrograde degeneration does not have a part in altering outer retinal layer's thickness. Some studies, however, show a thickening of the ONL + FR layer (and less pronounced of the INL+OPL) that may be mediated by the same retrograde degeneration mechanism. Vitreous traction has also been implicated but recently deemed unrelated.[14,15,16,17] Another plausible explanation can be the presence of the earlier mentioned retinal inflammation, with perivenous sheating and perivenous leakage, which occurs in eyes with AON within eight weeks of symptom onset.[5] This corroborates studies that refer to the dynamic changes occurring in deep retinal layers following AON, with a mean increased in ONL+FR layer at four months followed

by a decreased in retinal thickness at about 4-12 months and similar thickness comparing to baseline after four months.[17] Our results may be at this stage since none of the patients had had an episode of AON in the last year before OCT [11,18].

Our study has some limitations. Firstly we did not separate different MS types, which, in more aggressive types, can possibly have a more evident pathway of neuronal degeneration. Different follow-up times can also interfere with the results. Standard OCT techniques, although offering high resolution imaging, still cannot achieve cellular-resolution imaging. Therefore it is not possible to determine if the alterations detected in our study are due to activation of microglia cells (Müller cells in the retina) in response to ganglion cell death, hypertrophic neuronal cell bodies, alterations in subretinal fluids or a combination of these factors. Future studies, perhaps with adaptive optics technology, can possibly shed a light in this phenomenon.

OCT can currently be considered an imaging biomarker of global CNS atrophy for both monitoring neuronal degeneration and assessing non-invasively effectiveness of therapies that reduce neuroaxonal loss. We believe that ganglion cell atrophy that occurs so early in the course of MS is still undermined and could be reduced with valid neuroprotective therapies during therapeutic window using OCT technology as a primary or secondary outcome metric for MS patients.

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