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ARAŞTIRMA MAKALESİ

RETINAL NERVE FIBER LAYER ANALYSIS BY OPTICAL COHERENCE TOMOGRAPHY AND HEIDELBERG RETINAL TOMOGRAPHY: COMPARISON WITH VISUAL EVOKED POTENTIALS AND FUNCTIONAL DISABILITY IN MULTIPLE SCLEROSIS PATIENTS

MULTİPL SKLEROZ HASTALARINDA OPTİK KOHERANS TOMOGRAFİ VE HEIDELBERG RETİNAL TOMOGRAFİ İLE RETİNA SİNİR LİFİ TABAKASI ANALİZİ: GÖRSEL UYARTILMIŞ POTANSİYELLER VE FONKSİYONEL DİZABİLİTE İLE KARŞILAŞTIRMA

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ABSTRACT
Multiple sclerosis (MS) is a chronic disease which is characterised by widespread demyelinated lesions in the central nervous system (CNS). Disability from MS, is strongly associated with demyelination and axonal involvement. In MS patients, evaluation of retina by optic coherence tomography (OCT) and Heidelberg Retinal Tomography (HRT) methods gives us the possibility to visualize unmyelinated axons of the CNS directly. In this study, we aim to object to the relationship between retinal nerve fiber layer (RNFL) thickness and visual evoked potentials (VEP) and the disability scores in MS patients.
41 patients with MS and 20 healthy, age and sex matched volunteers were

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included. 82 eyes of 41 patients with MS and 40 eyes of 20 healthy individuals were assesed. In MS patients group, 8 of them had unilateral or bilateral previous optic neuritis at least 6 months before OCT. In MS patients and control group; the EDSS, VEP analysis, OCT and HRT measurements were performed.
The OCT and HRT measured RNFL thickness was statistically diminished significantly in MS patients compared to the control group. Furthermore, we showed a correlation between reduced RNFL values obtained with OCT and prolonged P100 latencies by VEP analysis and EDSS scale. On the contrary, there was no correlation between HRT measured RNFL thickness and P100 wave latencies.
All MS patients with or without previous optic neuritis showed RNFL atrophy measured by OCT and HRT. OCT and HRT could be used as an early structural biomarker of axonal damage in MS patients.
Keywords: Multiple sclerosis, retinal nerve fiber layer, visual evoked potentials, optic coherence tomography, Heidelberg retinal tomography.

ÖZET

Multipl Skleroz (MS), merkezi sinir sisteminin yaygın demiyelinizan lezyonları ile karakterize kronik bir hastalıktır. MS hastalarında ortaya çıkan özürlülük, demiyelinizasyon ve aksonal hasar ile yakından ilişkilidir. MS hastalarında, optik Koherans Tomografi (OKT) ve Heidelberg Retina Tomografisi (HRT) yöntemleri ile retinanın incelenmesi, bize merkezi sinir sisteminin miyelinsiz aksonlarını doğrudan değerlendirme imkanı sunar. Bu çalışma ile MS hastalarının retinal sinir lifi tabakası kalınlığı (RSLT) ile görsel uyartılmış potansiyeller (VEP) ve özürlülük arasındaki ilişkiyi ortaya koymak amaçlanmıştır.
MS tanılı 41 hasta ile yaş ve cinsiyete göre eşleştirilmiş 20 sağlıklı gönüllü çalışmaya dahil edildi. Toplam 41 MS hastasının 82 gözü ile 20 sağlıklı bireyin 40 adet gözü değerlendirildi. MS hasta grubunda, OKT'den en az 6 ay öncesinde tek veya iki taraflı optik nörit atağı geçiren 8 MS hastası vardı. Hasta ve kontrol grubunda; genişletilmiş özürlülük durum ölçeği (EDSS) hesaplandı, VEP analizleri, OKT ve HRT ölçümleri yapıldı.
MS hasta grubunda, OKT ve HRT ile ölçülen RSLT kalınlığı, kontrol grubu ile karşılaştırıldığında istatistiksel olarak anlamlı derecede azalmış olarak saptandı. OKT ile ölçülen RSLT kalınlığı ile VEP P100 değerleri ve EDSS arasında korelasyon saptadık.HRT ile yapılan RSLT kalınlığı ölçümü ve VEP P100 dalga latansı arasında ise benzer ilişki saptanmadı.
Optik nörit atağı olan ve olmayan tüm MS hastalarında, OKT ve HRT ile ölçülen RSLT kalınlığında azalma saptandı. OKT ve HRT, MS hastalarında aksonal hasarın erken yapısal biyomarkırı olarak kullanılabilir.

Anahtar Kelimeler: Multipl skleroz, retina sinir lifi tabakası, görsel uyartılmış potansiyeller, optik koherans tomografi, Heidelberg Retinal Tomografi

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system causing severe neurological disability in young adults. Unidentified environmental factors and susceptibility genes play a role in the pathogenesis leading to acute inflammatory injury of axons and glia, post-inflammatory gliosis and neuro degeneration. The clinical course is characterised by episodes with recovery, some leaving neurological deficits and followed by secondary progression.

Optic neuritis is one of the common causes of reversible vision loss which can occur as a manifestation of multiple sclerosis. Ocular imaging techniques such as optic coherence tomography (OCT) and Heidelberg retinal tomography (HRT) allows accurate analysis of the optic nerve head and the retina. Since there is evidence of early axonal damage in acute demyelinating optic neuritis, thinning of retinal layer measured by OCT may refer to direct

axonal loss in MS patients.
The aim of the present study was to evaluate the retinal nerve fiber layer (RNFL) thickness of patients with MS as markers of axonal loss in relation to the functional disability by comparing two different ocular imaging techniques. Furthermore, we object to determine the association between the thickness of the RNFL, visual evoked potentials (VEP) and functional disability in MS patients.

MATERIAL AND METHODS

Patients and controls:
We prospectively evaluated 50 MS patients who were regularly monitored in MS outpatient clinic in our hospital between 2009 and 2010 and 20 healthy individuals who were age and sex matched as controls. Inclusion criteria were the diagnosis of clinically definite MS according to the Mcdonald's criteria, irrespective of the disease subtype, absence of congenital disc abnormalities and refractive errors that can effect OCT and HRT measurements and presence of intraocular pressure less than 18 mmHg. Healthy individuals who admitted to our ophthalmology outpatient clinic were included as control group with normal intraocular eye pressure and normal neurologic and systemic examinations. From the 50 patients of the original cohort, we excluded nine patients: seven did not complete both OCT and HRT measurements, and two of them had glaucoma. In individual patients we performed the neurological and ophtalmologic examinations, and OCT and HRT studies in the same month. Our study protocol was approved by local ethics committee, Izmir Regional Ethical Committee No:2. The subjects in the patient and control groups assigned the informed consent according to the Declaration of Helsinki before entering the study.
OCT Analysis:
The thickness of the RNFL was measured in both eyes using optic coherance tomography with a Stratus OCT (StratusOCT 3000, and OCT 4.0 software, Carl Zeiss, Dublin) with 3,4 mm fast scan protocol. The OCT software employed an automated computarized algorithm to calculate the average thickness of the inner RNFL. The average and quadrant (temporal, superior, inferior and nasal) RNFL thickness were obtained in μm. A trained medical ophthalmic technician performed all OCT testing at Ophthalmology Department, Dokuz Eylul University, School of Medicine.
HRT analysis:
HRT 3 scanner (Heidelberg Eye Explorer software version 1.5.1, Heidelberg Engineering, GmbH, Heidelberg, Germany) was performed by an ophthalmic technician. A software programme calculated various parameters including RFNL thickness. All HRT testing was performed at Ophthalmology Department, Dokuz Eylul University, School of Medicine.
VEP analysis:
VEPs were recorded with a Medelec Sapphire 4ME machine, in a dark room of our electrophysiology unit. Electrodes applied to the scalp and positioned over the mid-occipital and mid-frontal locations, with Cz as a ground. After occlusion of one eye, stimulation was monocular and visual stimuli followed a checkerboard pattern arrangement with a reversed contrast of 1 Hz. P100 wave's peak to peak amplitude and latency were measured at least twice and the average was considered.
EDSS scale:
Expanded disability status scale (EDSS) was performed by experimented neurologists at MS outpatient clinic.
Statistical Analysis:
All data analyses were performed using the SPSS software version 17.0. Kolmogorov-Smirnov test was used to assess the normal distribution of all variables. For parametric variables, the differences between groups were assessed with the t-test and for the nonparametric variables we used Mann-Whitney U test. To assess whether a correlation exists between OCT, VEP and EDSS score, linear regression analysis (Pearson's test) was adopted. Linear regression analysis (Pearson's test) was also performed between HRT, VEP and EDSS score to assess relationships among test results. In all statistical analyses, $p<0,01$ was considered as significant.

RESULTS

A total of 61 subjects were included in the study, 41 MS and 20 healthy individuals as controls. Demographic characteristics and RNFL values for all subjects are summarized in Table-1.
We compared the average RNFL thickness between healthy eyes and MS eyes with and without a history of optic neuritis (Table-1). Both OCT and HRT techniques detected significant differences between MS patients and healthy controls. OCT measured average RNFL thickness was 93.75 ± 17.8 µm in MS patients and 108.2 ± 9.49 µm in the control group (p=0,000004). HRT measured average RNFL thickness was 0.232 ± 0.066 mm in MS patients and 0.267 ± 0.028 mm in the control group (p= 0,0001). We found that the average RNFL and the RNFL values in temporal, superior and inferior quadrants obtained with OCT were lower and the difference was statistically significant in MS patients when compared with the control group (Table-2).

	MS patients (n: 82 eyes)	Control patients (n: 40 eyes)
Age (years) (mean, (standard deviation))	39.6 (9.96)	35.3 (6.6)
Male / Famale	9/32	5/15
ON(+) / ON(-)	12/70	□
EDSS scale (mean) (min-max)	3.08 ± 1.1 (1-6)	□
P100 wave latency	122,8 ± 19,36 (95,4-195)	□
RNFL (average) OCT (µm) all eyes	93.75 ± 17.8	108.2 ± 9.49
RNFL (average) HRT (mm) all eyes	0.232 ± 0.066	0.267 ± 0.028

Table 1. Summary of the multiple sclerosis and healthy subject cohorts.

We also examined the capacity of OCT and HRT to detect RNFL atrophy measured in MS patients with or without optic neuritis. OCT measured average RNFL thickness in MS group with a previous optic neuritis attack (MS-ON) was 83.85 ± 22 (39 - 111.5) µm and with no history of optic neuritis (MS-NON) was 95.45 ± 16.62 (51-129.29) µm. We found that in MS-ON group, the average RNFL and the thickness of the temporal, nasal, superior and inferior RNFL quadrants were lower when compared with MS-NON group (Table-3). Also we found that average RNFL thickness obtained with HRT was 0.2 ± 0.03 (0.15 -0.24) mm in MS-ON group and 0.23 ± 0.069 (0.07 – 0.41) mm in MS-NON group. The results of OCT and HRT measurements of the RNFL decreased in all patients with MS, but a nonsignificant difference was seen between MS-ON and MS-NON groups. Therefore, although both techniques provide information about the changes in the RNFL in MS patients, there was no significant difference between MS-ON and MS-NON groups (Table-3).
There was a significant correlation between average RNFL obtained with
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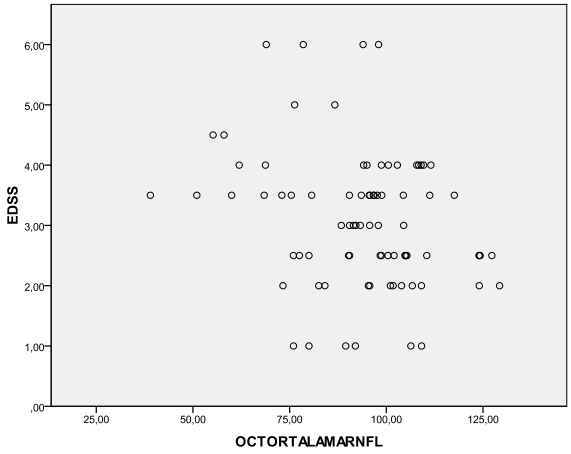

Figure 1. The average RNFL thickness measured by OCT plotted against EDSS score in MS patients

OCT and EDSS score among MS patients (p<0,001) (Figure-1). The correlation between VEP P100 latency and average RNFL thickness measured by OCT was also significant (p<0,001) and it is presented in Figure-2. There was no correlation between HRT measured RNFL thickness and functional diasability as measured by EDSS score (p=0,253). Also, no correlation was found between VEP P100 latency and RNFL atrophy measured by HRT (p=0,44). In addition to that the RNFL atrophy measured by OCT and HRT showed no correlation with

EDSS score and VEP P100 latency betwen MS-ON and MS-NON groups.

OCT	MS (N=82 eyes)	Controls (N=40 eyes)
average RNFL	93,75*	108,22
temporal RNFL	58,93*	82,12
superior RNFL	118,26*	134,65
nasal RNFL	79,57	82,37
inferior RNFL	117,30*	133,52

Table 2. Differences in OCT variables between patients with MS and healthy controls. The RNFL results are expressed as the mean in μm. * p<0,01.

	MS-ON (N=12 eyes)	MS-NON (N=70 eyes)	p-value
OCTaverage RNFL	83,8	95,4	0,03
OCT temporal RNFL	52,91	59,97	0,2
OCT superior RNFL	111,4	119,4	0,2
OCT nasal RNFL	64	82,2	0,02
OCT inferior RNFL	106	119,2	0,12
HRT average RNFL	0,2	0,23	0,07

Table 3. RNFL values in MS patients with and without optic neuritis measured by OCT (expressed in μm) and HRT(expressed in mm). MS-ON, MS patients with optic neuritis; MS-NON, MS patients without optic neuritis.

DISCUSSION

The retina is a spesific compartment of the central nervous system that has been a target by the disease process in MS patients. As retina contains axons and glia absent of myelin, it is ideal for visualizing the neurodegeneration to understand mechanisms of tissue injury in MS patients. Ocular imaging technologies, OCT and HRT, which have been recently used in the analysis of optic neuropathies and neurodegenerative diseases like MS, allow an quantative and reproducible measurements of the optic nerve head and the RNFL. OCT can provide investigators precise information about the structural composition of the retina in tracking the progress of neurodegeneration. OCT and HRT are promising new tools to investigate the pathophysiology in MS and optic neuritis. Optic neuritis is an inflammatory optic neuropathy which is presented in multiple sclerosis patients about %20 as a first symptom (1,2). There is a high risk of developing MS in the future in patients who experienced an episode of optic neuritis (3). In the present study we aim to assess whether RNFL measurements provided by OCT and HRT were useful markers of axonal loss in MS patients. We also compared these two techniques and evaluated the relationship between RNFL thickness, functional disability and VEP P100 latency.

The average RNFL thickness in MS patients measured by OCT is noted to be 95.45 ± 16.62 μm in unaffected eyes, a mean of 83.85 ± 22 μm.in eyes affected by previous optic neuritis and 108.2 ± 9.49 μm in healthy controls. In this study, MS patients with previous optic neuritis had a thinner average RNFL thickness than the rest of MS patients without optic neuritis. Also, all patients in MS group had a thinner average RNFL than in healthy controls. In the literature, the OCT measured RNFL values are found to be diminished among patients with optic neuritis and MS patients (4,5,6,7,8,9,10,11,12,13,14).

In 1999, Parisi et al. reported the earliest application of OCT technology in 14 patients with MS who had previous event of optic neuritis (4). The thickness

of the RNFL was shown to be reduced in the affected eyes of the patients with MS versus the eyes of controls. Even in the unaffected eyes of MS patients, the reduced RNFL thickness was noted when compared to the control group. In our study we found similar results in patients with MS and optic neuritis. Both MS groups had thinner RNFL than healthy individuals. OCT measured RNFL thickness was significantly reduced in MS patients and we found a decrease in all quadrants although the decrease was smaller in the nasal quadrant. In Fisher, et al. study (6), they found similar findings. Also Parisi et al. found no correlation between VEP parameters and RNFL thickness in MS patients previously affected by optic neuritis (4). We observed that there was no correlation between VEP P100 latency and RNFL thickness measured by OCT and HRT in MS patients with optic neuritis.

Trip and colleques reported their results with OCT technology in MS and clinically isolated syndrome patients with optic neuritis in 2005 (5). They found an average of %33 reduction in RNFL thickness in the affected eyes of patients when compared with eyes of controls. In addition, they showed that axonal integrity is better correlated with VEP P100 amplitudes and the RNFL thinning measured by OCT. Although Parisi (4) and Trip (5) found no correlation between VEP P100 latency and RNFL measurements, our study demonstrated that the RNFL thickness is strongly correlated with prolonged VEP P100 latency in MS patients. However, Pueyo et al. detected a correlation between P100 and RNFL thickness in MS patients (15).

The Balcer study, suggested that the unaffected eyes of patients with MS who have a history of acute optic neuritis are at a similar risk for axonal loss to the eyes of patients with MS in general (6). In the present study we showed that both MS groups have more axonal loss than do age and sex matched healthy controls.

In MS patients, axonal damage is responsible for permanent disability. Fisher et al. described a correlation between the RNFL thickness and functional impairment measured by EDSS (6). Pueyo and colleques stated that there was a significant but poor correlation between VEP P100 latency and EDSS while no correlation between global EDSS and RNFL atrophy was found (15). In our study, we observed that the RNFL thickness measured by OCT is strongly correlated with P100 latency and EDSS. Toledo et al. also found a correlation between physical disability as measured by EDSS and the RNFL thickness obtained with OCT (16). Despite the significant difference with OCT, HRT showed no correlation with P100 latency and EDSS scores.

The average RNFL thickness in MS patients measured by HRT was reduced when compared to the control group. Although the average RFNL was thinner in MS patients with optic neuritis than patients without optic neuritis, statistically there was no significant difference among MS patients.

While OCT provides a direct measure of RNFL thickness, HRT does an indirect measurement. For this reason, OCT provides information about axonal loss and HRT is more sensitive to volume changes in the tissue. Laron et al., concluded that OCT is a valuable tool in documenting axonal loss (17).

In conclusion, we documentated the RNFL measurements in patients with MS and compared the results between the patients with optic neuritis and without optic neuritis. We found that RNFL thickness obtained with OCT is strongly in association with VEP P100 latency and EDSS score. In addition, our results indicate that the RNFL is also thinner in MS patients without optic neuritis, too. However, future studies with a larger population is needed.

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