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Original Article / *Özgün Araştırma*

Is Single Measurement Enough to Get a Reliable Result with Optical Coherence Tomography?

Optik Koherens Tomografi ile Güvenilir Sonuç Almak için Tek Ölçüm Yeterli mi?

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Summary

Purpose: To evaluate the repeatability and reliability of retinal nerve fiber layer (RNFL) thickness measurements using optical coherence tomography (OCT).

Material and Method: Two hundred sixty-six eyes of 135 subjects (glaucoma, glaucoma suspects and healthy) were included in this study. Three sequential inferior, superior, nasal and temporal RNFL thickness measurements were performed using Spectral OCT [Opko/OTI, Inc., Miami, FL] by one operator at one session without pupillary dilatation. The differences between these three measurements of each quadrant in each eye were compared in microns and percentages. Repeated measures analysis of variance was performed for statistical analysis. Reliability is measured by intraclass correlation coefficient (ICC) for each quadrant.

Results: ICCs of all quadrants ranged between 0.77 and 0.92, with the measurements of nasal quadrant being the least reproducible and the inferior being the most reproducible of all quadrants. RNFL measurement errors over 20% were seen in 9.63% of nasal quadrant, 5.3% of temporal quadrant, 0.6% of superior quadrant and only 0.3% of inferior quadrant measurements.

Discussion: In order to get more repeatable and reliable results with OCT, sequential measurements more than one should be considered. We believe that special attention is required in the analysis of data of nasal and temporal quadrants. *(Turk J Ophthalmol 2012; 42: 11-5)*

Key Words: Glaucoma, optical coherence tomography, reliability, repeatability, retinal nerve fiber layer

Özet

Amaç: Optik koherens tomografi (OKT) ile retina sinir lifi tabakası (RSLT) kalınlığı ölçümlerinin tekrarlanabilirliğini ve güvenilirliğini değerlendirmek.

Gereç ve Yöntem: Sağlıklı, glokom tanısı veya şüphesi olan 135 kişinin 266 gözü çalışmaya katıldı. Aynı seansta, aynı kişi tarafından, pupil dilate edilmeden, OKT [Opko/OTI,Inc.,Miami,FL] cihazı ile inferior, superior, nazal ve temporal RSLT kalınlığı ardışık olarak 3 kez ölçüldü. Her gözün her kadranının üç ölçümü arasındaki farklar mikron ve yüzde cinsinden karşılaştırıldı. İstatistiksel analiz için 'Tekrarlı ölçümlerde varyans analizi testi' kullanıldı. Güvenilirlik 'Sınıf içi korelasyon katsayısı'(SKK) ile değerlendirildi.

Sonuçlar: Tüm kadranların SKK'sı 0,72 ile 0,92 arasında olup, nazal kadran ölçümleri en az tekrarlanabilir, inferior kadran ölçümleri ise tüm kadranlar içinde en tekrarlanabilir ölçümler olarak bulundu. Nazal kadran RSLT kalınlığı ölçümlerinin % 9,63'ünde, temporal kadran ölçümlerinin %5,3'ünde, süperior kadran ölçümlerinin %0,6'sında, inferior kadran ölçümlerinin ise sadece %0,3'ünde %20'den fazla hata oranı görüldü.

Tartışma: OKT ile tekrarlanabilir ve daha güvenilir sonuçlar alabilmek için, ardışık olarak birden fazla ölçüm yapılması düşünülmelidir. Nazal ve temporal kadran verilerinin analizinde daha fazla özen gösterilmesi gerektiğine inanmaktayız. *(Turk J Ophthalmol 2012; 42: 11-5)*

Anahtar Kelimeler: Glokom, optik koherens tomografi, tekrarlanabilirlik, güvenilirlik, retina sinir lifi tabakası

Introduction

Optical coherence tomography (OCT) is a noninvasive, noncontact, transpupillary imaging method that can provide cross-sectional tomographic images of retinal structures. The probe beam is directed into the eye and the resections from tissue interfaces give information about the distances and thicknesses of the ocular structures.[1] Early studies revealed that the

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measurements of retinal nerve fiber layer (RNFL) thickness appear promising in monitoring glaucomatous changes.[1,2] However, in order for this new technology to be introduced into the routine practice, its repeatability and reliability must be established. Many authors have reported on OCT reproducibility and have demonstrated standard deviations (SD) of RNFL and retinal thickness measurements within the range of approximately 10-20 microns (10-20%) in normal and in glaucomatous eyes.[3-6]

This study was designed to evaluate RNFL thickness differences in each patient measured by one observer 3 times sequentially, using Spectral OCT, and to assess the reliability and the repeatability of these measurements.

Method

One hundred sixty-two eyes of 82 females and 104 eyes of 53 males (a total of 266 eyes) of 135 subjects (glaucoma, glaucoma suspects and healthy) were included in this study. After facilitating proper alignment of patients' forehead and chin, three sequential measurements of RNFL thickness in the inferior, superior, nasal and temporal quadrants were done by Spectral OCT {Opko/OTI, Inc., Miami, FL} by one operator at one session without pupillary dilatation, in a dimly lit room. The differences between these three measurements in each retinal quadrant of each eye were calculated in microns and also converted to percentages, as the measurements of the thicker quadrants (superior, inferior) would not be affected at the same rate as the thinner quadrants (nasal, temporal) would do.

The mean age of the participants was 43.2±14.7 (range: 16-78) years. Participants without a history of eye surgery and with a visual acuity of 0.2 logMAR (6/9 Snellen) or better were included in the study.

All participants gave informed consent and the study was conducted according to the tenets of the Declaration of Helsinki. The data of the study were transferred to SPSS 15.0 Packet programme and analyses were done by this programme. Repeated measures analysis of variance was used for statistical analysis. P value below 0.05 was accepted as an indicator for statistical significance. Intraclass correlation test was used to asses the reproducibility of the measurements. The intraclass correlation coefficient (ICC), 95% confidence interval (95%CI) and coefficient of variation (CV) were calculated. The cut-off value of confidence interval (CI) for ICC accepted for a good reproducibility was over 0.70.

Table 1. Intraclass Correlation Coefficient, 95% Confidence Interval and Coefficient of Variation values of 3 sequential RNFL measurements in four quadrants. Although the measurements of nasal quadrant with an ICC of 0.77 indicate good reprodubility, it is seen that nasal quadrant measurements are less reproducible compared to other 3 quadrants

	ICC	95% CI	CV (%)
Inferior quadrant	0.92	0.90-0.93	14.36
Superior quadrant	0.91	0.89-0.93	14.53
Nasal quadrant	0.77	0.72-0.81	16.72
Temporal quadrant	0.83	0.80-0.86	17.51

ICC: Intraclass Correlation Coefficient; 95%CI: 95% Confidence Interval; CV: Coefficient of Variation = (Standard Deviation/mean) X 100

Results

The mean values for each quadrant were as follows: inferior (133.5±19.5 μ), superior (129.0±18.7 μ), nasal (88.7±16.9 μ), and temporal (72.7±13.9 μ).

ICCs, %CI values and CVs of 4 quadrants are presented in Table 1. ICCs of all four quadrants were excellent with inferior and superior quadrants having the highest ICCs (0.92;0.91), while temporal quadrant's ICC was lower (0.83) and nasal quadrant's ICC being the lowest (0.77). However, all ICCs being over 0.70, indicate excellent reproducibility of all measurements.

Although the differences between the mean values of each quadrant for all 3 repeated scans were not found to be statistically significant (Table 2 and Figure 1), 15,8% of RNFL measurements showed errors over 20% (9.6% in nasal; 5.3% in temporal; 0.6% in superior and 0.3% in inferior quadrants).

The numbers and percentages of measurements in eyes having differences below 4.99%, between 5-9.99%, between 10-19.99%, and over 20% are shown quadrant by quadrant in Table 3 and Figure 2, where the first scan is compared to the second and to the third one and the second to the third scan.

Table 2. The mean values for each quadrant were found to be statistically not significant. This can be explained by the fact that underestimated and overestimated measurements neutralized each other

OCTI	Mean±SD	Median (Min-Max)	p*
OCT1i	133.5±19.5	134.0 (57.0-194.0)	
OCT2i	133.0±19.8	133.0 (58.0-123.0)	0.61
OCT3i	133.3±19.7	133.0 (56.0-186.0)	
OCTS			
OCT1s	129.0±18.7	130.0 (67.0-187.0)	
OCT2s	129.9±19.9	131.0(67.0-183.0)	0.18
OCT3s	129.4±19.4	131.0(71.0-186.0)	
OCTN			
OCT1n	88.7±16.9	89.0 (48.0-142.0)	
OCT2n	88.9±15.5	89.0(43.0-129.0)	0.93
OCT3n	88.8±16.0	88.0(47.0-137.0)	
OCTT			
OCT1t	72.7±13.9	72.0(36.0-116.0)	
OCT2t	71.5±13.1	71.0(34.0-121.0)	0.05
OCT3t	72.0±13.1	72.0(35.0-116.0)	

OCT; Optical coherence tomography, SD; standard deviation, min; minimum, max;maximum, p*: statistical significance <0.05, (Repeated-Measures test), i;inferior, s;superior, n;nasal, t;temporal,

Table 3. Measurement errors over 20% between 3 scans were seen especially in the nasal quadrant, 29 (11%), 28 (11%) and 20 eyes (8%), respectively. The errors in the temporal quadrants were relatively higher compared to superior and inferior quadrants

Errors in %	**OCT**		**OCT**		**OCT**		**OCT**		**OCT**		**OCT**		**OCT**		**OCT**		**OCT**		**OCT**	
between sequential scans	**1I-2I**		**1I-3I**		**2I-3I**		**1S-2S**		**1S-3S**		**2S-3S**		**1N-2N**		**1N-3N**		**2N-3N**		**1T-2T**		**1T-3T**		**2T-3T**	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
≤ 4.99%	175	66	173	65	173	65	168	63	158	59	181	68	98	37	90	34	94	35	118	44	108	41	131	49
5% - 9.99%	68	26	61	23	64	24	68	26	73	27	62	23	69	26	79	30	86	32	76	29	91	34	69	26
10% - 19.99	22	9	32	12	28	11	26	10	34	13	23	9	70	26	69	26	66	25	59	22	48	18	56	21
over 20%	1	0	0	0	1	0	4	2	1	0	0	0	29	11	28	11	20	8	13	5	19	7	10	4

OCT;optical coherence tomography, n;number, I;inferior, S;superior, N;nasal, T;temporal, %;percentage

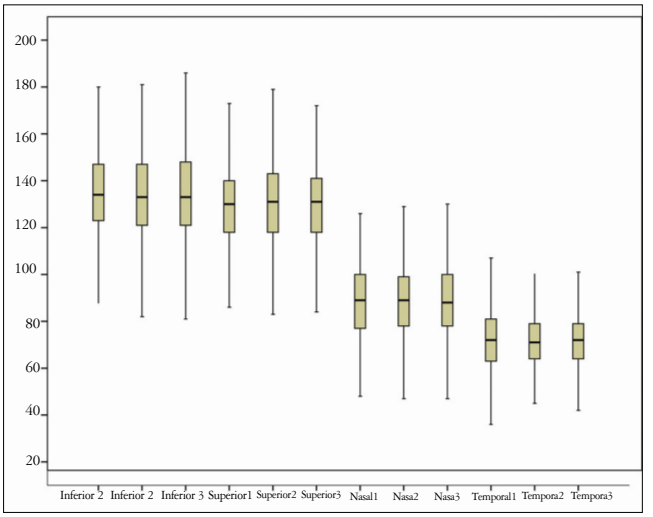

Figure 1. Although the errors between three scans according to the mean values of each quadrant were not statistically significant, when comparing all measurements one by one, deviations were leading up to 43 microns (83%)

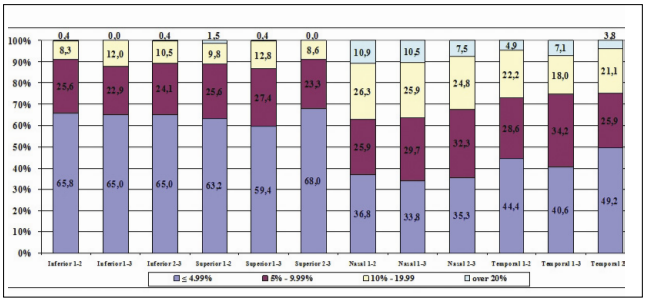

Figure 2. While in most of the superior and inferior quadrant measurements error rates were below 5% (colour blue); green-coloured areas representing errors over 20% were dominantly detected in temporal and nasal quadrants

Discussion

OCT has an ability to provide quantitative measurements of internal ocular structures. It may be used for retinal and macular pathology diagnostics as well as for RNFL evaluation in glaucoma.[3] It is found to have an excellent reproducibility and sensitivity for the diagnosis of glaucoma in many studies.[4-9] OCT measurements of RNFL thickness correlates with functional status of the optic nerve, as measured by visual field examination,[3] and it appears promising as a tool for early diagnosis of glaucoma. It has been shown that in 60% of perimetric glaucoma eyes, there was already an evidence of alteration in the RNFL 6 years before the occurrence of the visual field defect.[3] Therefore, accurate assessment of such changes is of great importance for both early diagnosis and monitoring of patients.

Time-domain (TD) and Spectral-domain (SD) OCT use the same basic working principles; however, the scan rate of SD-OCT is at least 18,000 axial scans per second, with an improved axial resolution of 5 µm, while TD-OCT collects 400 axial measurements per second with an axial resolution of approximately

10 µm enabling a standard 512 A-scan image in approximately 1.3 seconds (10). In TD-OCT, the depth data of the retina is collected as a function of time by moving the reference mirror. On the contrary, the reference mirror in SD-OCT is stationary, which makes SD-OCT more sensitive and accurate. Although we used SD-OCT, we got very inconsistent measurements up to 83% difference, especially in the temporal and nasal quadrants.

Repeatability is the variability of measurements by the same operator measuring the same entity, under the same conditions within a short period of time. It is a measure of the precision of the instrument. SD of the repeated measurements is a measure of repeatability.[11]

Reliability, as measured by ICC, demonstrates the reproducibility of measurements of the same entity, by the same operator. As we encountered some inconsistency between RNFL measurements in scans of some of our patients, which obscured the monitoring of the course of glaucoma, we decided to design this study. As a consequence of this inconsistency that we observed in our patients, minimum of 3 OCT scans from each patient are performed as a routine practice in our clinic, and the mean value of them is taken into account while evaluating the data.

Although the sensitivity of Stratus OCT is reported to be as 77.2 to 89 % , and the specificity, as 74 to 92% in previous reports by various authors, it is accepted that OCT is open to some errors and its results may show variability.[9,12-14] For the best quality of the scan, a good centration around the optic disc is needed to obtain the exact measurement of peripapillary RNFL thickness as recommended by the manufacturer company.

Possible artifacts and variability such as medium opacity,[15,16] pupil dilatation,[7] type of scan,[7,8] the quadrant measured,[8] sampling density and the number of the scans performed,[17] different generations of OCT machines and software,[18,19] corneal dryness,[20] optic nerve head size[21] and normative database[22] may affect the reliability of the data obtained. The centration of the scan around the optic disc is assessed with the help of the scanning ring subjectively, which may lead to a wide range of eccentricities. In a study investigating the effect of eccentric scans on RNFL measurement, it was claimed that only the inferiorly eccentric scans produced data similar to the concentric ones, while superiorly, nasally and temporally eccentric scans showed significantly different results.[23] In our study, especially the data obtained from temporal and nasal quadrants were found to be inconsistent. As the optic disc has a slightly vertically oval form with the vertical diameter being about 7-10% larger than the horizontal, we speculate that the thinner space without RNFL between vascular structures as well as the temporal and nasal quadrants compared to the superior and inferior quadrant area may not allow accurate measuring in all attempts (24. In addition, depending on the size of the optic disc, the distance from the circular scan to the disc margin varies, and the RNFL becomes thinner as it gets away from the disc margin, which may result with some errors when measuring the thickness.

A study evaluating RNFL measurements using OCT 2000 (Humphrey Zeiss Instruments) indicated that 5 scans may be needed to produce optimum repeatability.[25] Schuman et al. performed RNFL measurements with OCT in 21 normal and glaucomatous subjects five times in a 1-month period and found ICCs between 0.42 and 0.61, concluding that RNFL thickness measurements are reproducible(4). Paunescu et al. reported about the reproducibility of the Stratus OCT in 10 normal subjects scanned 6 times per day on 3 different days over a 5-month period. They yielded lower ICC values, which may indicate that there may be variation in one and the same person on different days.[7] Although OCT seems to be a reliable and repeatable technique for the measurement of RNFL according to the statistical results and past studies (12,26), we found that 9.6% of measurements of nasal, 5.3% of measurements of temporal, 0.6% of measurements of superior and 0.3% of measurements of inferior quadrants had thickness differences out of 20% margin of error. This may suggest that the measurements of nasal and temporal quadrants in nearly 10% of eyes differed from the other 2 previous scans with a ratio of 20%, which makes the monitoring of these quadrants untrustable. In a study by Blumenthal et al.,[27] RNFL thickness measurements were also reproducible in the same session, with nasal quadrant being the least reproducible quadrant as in our study, while the temporal quadrant was the most reproducible differentiating from our study.

Due to numerous errors between the scans, taking more than one scan in all patients and getting the mean values of each quadrant to discard extreme measurements especially in the nasal and temporal quadrants may be beneficial. We recommend that careful attention should be paid when measuring and evaluating RNFL thicknesses, especially in the nasal and temporal quadrants, by means of reliability. Further studies are needed to standardize OCT measurements.

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