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Reproducibility of peripapillary retinal nerve fiber layer thickness measurements using Spectral Domain OCT in Brazilian patients

Reprodutibilidade da espessura da camada de fibras nervosas da retina utilizando-se o Spectral Domain OCT em pacientes brasileiros

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ABSTRACT

Purpose: To evaluate the reproducibility of peripapillary retinal nerve fiber layer (RNFL) thickness measurements in normal eyes and eyes with glaucoma using spectral domain optical coherence tomography (SDOCT).

Methods: One eye of 79 normal and 72 glaucoma patients was analyzed. All patients underwent a complete ophthalmological examination, including visual acuity testing; intraocular pressure, slit-lamp examination, indirect ophthalmoscopy; and the glaucoma group underwent achromatic perimetry with the 24-2 SITA Fast Humphrey Field Analyzer. All patients' eyes were scanned using the spectral domain optical coherence tomography - Spectralis® and one of them was chosen randomly. Three consecutive circular B-scan centered at the optic disc were performed in one visit.

Results: The intraclass correlation coefficient (ICC), coefficient of variation and test-retest variability for the mean retinal nerve fiber layer thickness were respectively: 0.94, 2.56% and 4.85 µm for the normal group and 0.93, 4.65% and 6.61 µm for the glaucomatous group. The intraclass correlation coefficient for retinal nerve fiber layer thickness in all quadrants were all excellent in both groups, with the superior quadrant having the highest ICCs (0.964) in glaucomatous eyes and nasal quadrant measurements having the lowest (0.800), but still excellent in eyes without glaucoma. The coefficient of variation was between 2.56% - 8.74% and between 4.65% - 11.44% in normal and glaucomatous group respectively. The test-retest variability was between 4.85 µm and 11.51 µm in the normal group and between 6.61 µm and 14.24 µm in the glaucomatous group. The measurements in glaucomatous eyes were more variable than normal eyes.

Conclusions: Spectral domain optical coherence tomography showed excellent reproducibility with regard to retinal nerve fiber layer thickness measurements in normal and glaucomatous eyes.

Keywords: Diagnostic techniques, ophthalmological; Glaucoma/diagnosis; Tomography, optical coherence/methods; Retinal ganglion cells; Optic nerve/pathology; Nerve fibers; Reproducibility of results

RESUMO

Objetivo: Avaliar a reprodutibilidade da medida da espessura da camada de fibras nervosas da retina (CFNR) em olhos sem e com glaucoma utilizando-se tomografia de coerência óptica de domínio espectral (spectral domain OCT - SDOCT).

Métodos: Foram analisados apenas um olho de 79 pacientes normais e 72 com glaucoma. Todos os pacientes realizaram um exame oftalmológico completo, incluindo acuidade visual, pressão intraocular, biomicroscopia, oftalmoscopia indireta e, para o grupo com glaucoma, perimetria acromática 24-2 SITA Fast Humphrey Field Analyzer. Foram realizados em todos os olhos e em apenas uma visita, três B-scans circulares centrados no disco óptico utilizando-se o SDOCT - Spectralis®.

Resultados: O coeficiente de correlação intraclasse (ICC), coeficiente de variação e variabilidade teste-reteste para a média de espessura da camada de fibras nervosas da retina foram respectivamente: 0,94, 2,56% e 4,85 µm para o grupo sem glaucoma e 0,93, 4,65% e 6,61 µm para o grupo glaucomatoso. O coeficiente de correlação intraclasse foi excelente em ambos os grupos em todos os quadrantes, com o quadrante superior sendo o maior (0,964) no grupo glaucomatoso e o nasal sendo o menor (0,800), mas ainda excelente, em olhos sem glaucoma. O coeficiente de variação foi entre 2,56% - 8,74% e entre 4,65% - 11,44%, nos grupos sem e com glaucoma, respectivamente. A variabilidade teste-reteste variou de 4,85 µm e 11,51 µm no grupo sem glaucoma; e entre 6,61 µm e 14.24 µm no com glaucoma. Olhos com glaucoma apresentaram-se mais variáveis que os sem glaucoma.

Conclusão: A tomografia de coerência óptica "spectral domain" apresentou excelente reprodutibilidade da espessura da camada de fibras nervosas da retina em pacientes sem e com glaucoma.

Descritores: Técnicas de diagnóstico oftalmológico; Glaucoma/diagnostico; Tomografia de coerência óptica/métodos; Células ganglionares da retina; Nervo óptico/patologia; Fibras nervosas; Reprodutibilidade dos testes

INTRODUCTION

Glaucoma is an optic neuropathy, multifactorial, characterized by accelerated death of the retinal ganglion cells, subsequent axonal loss, and visual field impairment[1].

Detected defects in the retinal nerve fiber layer (RNFL) years before arising alterations in the visual field suggests that anatomical alterations of the RNFL can identify progression of the glaucoma before it causes functional loss in the visual field[2].

Methods for reliably establishing glaucomatous nerve atrophy are limited. Optical coherence tomography (OCT) is a noninvasive, high-resolution imaging technique that allows in vivo measurements of RNFL in cross section. High-resolution imaging of retinal structure is clinically relevant for the diagnosis of glaucoma[3].

Until recently, the OCT commercially available was the time domain OCT (TDOCT), which provides an axial resolution of 10 µm, and cross sectional retinal images consisting of 512 A-scans can be acquired

in 1.28 seconds. The main disadvantage of TDOCT technology is the limited resolution and slow acquisition time. With the spectral domain OCT (SDOCT) or Fourier domain, the echo time delays of light are measured by acquiring the interference spectrum of the light signal and converted to depth information by Fourier transform[4].

The detection method using a spectrometer allowed a considerable increase in imaging speed and resolution without compromising image quality[5]. The increase in imaging speed minimizes motion artifacts. Resolution is up to 5 times higher, and imaging speed is 60 times faster than in conventional time domain OCT[4].

Estimate reproducibility of RNFL thickness using OCT is essential for diagnostic precision and in particular, describes the smallest changes detectable for identifying and monitoring the progression of glaucoma as well as indicates therapeutic interventions[6,7].

Quantifying the reproducibility of the RNFL thickness measurements using Spectralis® spectral domain OCT is an important step in evaluating the potential usefulness of this device for the diagnosis of glaucoma and for determining glaucomatous progression.

To our knowledge the present study is the first to report on the reproducibility and test-retest variability of RNFL thickness measurements using Spectralis® SD-OCT device in Brazilian population.

The purpose of this study was to evaluate the reproducibility of peripapillary retinal nerve fiber layer (RNFL) thickness measurements in normal and glaucomatous eyes using spectral domain optical coherence tomography (Spectralis®) in Brazilian population.

METHODS

This prospective observational cross-sectional study was approved by the Institutional Review Board / Ethics Committee of the University of Brasília, Brasília, Brazil. All participants in this study gave their written informed consent. All normal and glaucoma participants were enrolled in this study through the glaucoma and cataract service of Brasília Center of Vision (CBV, Brasília, Brazil) between August and December 2010.

A total of 151 eyes from 79 normal patients and 72 patients with moderate to advanced glaucoma were analyzed. All subjects underwent a complete ophthalmological examination, including medical and family history; visual acuity testing with refraction; intraocular pressure measurements using Goldmann applanation tonometry (GAT), a complete slit-lamp examination, including indirect ophthalmoscopy. Patients from the glaucoma group underwent achromatic perimetry using 24-2 SITA FAST Humphrey Field Analyzer (Humphrey- Zeiss Systems, Dublin CA).

Inclusion criteria for both groups were: age more than or equal to 40 years, spherical refractive error less than or equal to 5 diopters (D), cylindrical refractive error less or equal to 3D.

Inclusion criteria for normal subjects were: best-corrected visual acuity of 20/60 or better; normal slit-lamp examination; intraocular pressure of 21 mmHg or less; normal appearing optic nerve heads; and no history of ocular surgery or laser treatments.

Glaucoma patients were defined on the basis of having either:
1. An abnormal Humphrey Field Analyzer, defined as having MD less than -12dB; Less than 50% of the points are depressed bellow the 5% level and less than 20 points are depressed bellow the 1% level on the patterns deviation plot; No points in the central 5° can have a sensitivity of 0 dB; Only one hemifield may have a point with sensitivity of < 15 dB within 5° of fixation[8] and
2. Glaucoma optic disc change defined as a cup-to-disc ratio greater than or equal to 0.6, cup-to-disc ratio asymmetry between the eyes greater than or equal to 0.2, disc rim thinning, notching, localized pallor or nerve fiber layer defect [9].

All patients from the glaucoma group had primary open-angle glaucoma or chronic angle-closure glaucoma, being excluded any other kind of glaucoma.

Exclusion criteria for both groups included other intraocular diseases, as well as diseases that affect the visual field, like pituitary lesion, diabetes, retinal conditions or secondary cause of intraocular pressure increase.

Patients were excluded in the glaucoma group if they had unreliable automated perimetry results: fixation loss, false positives or false negatives more than 33%. Visual fields were carried out at least twice[9].

OCT MEASUREMENTS

All patients' eyes were scanned using the commercially available SDOCT Spectralis® HRA (Heidelberg Retina Angiograph) + OCT (Heidelberg Engineering). This instrument uses a wavelength of 820 nm in the near infrared spectrum in the SLO (scanning laser ophthalmoscopy) mode. The light source of the SDOCT is a super luminescent diode with a wavelength of 870 nm. Infrared images and OCT scans (40,000 A-Scan/sec) of the dual laser scanning systems are acquired simultaneously[10]. Three consecutive circular B-scan (3.4-mm diameter, 768 A-scans) centered at the optic disc were performed in one visit by the same operator. The scanning circle was centered manually on the optic disc first, although the participant was looking at the internal fixation light. The RNFL borders could be clearly identified and were marked automatically by the segmentation software. Images were judged to be of sufficient quality on the basis of subjective operator evaluation. Within each scan session, the instrument alignment and controls were not changed, unless as part of image acquisition process. The subject was repositioned between the scan measurements only when necessary. There was no specific attempt to reposition the subjects between scans done during the same session. Images were acquired through undilated pupils.

The Spectralis® reports the average RNFL thickness in the superior, temporal, inferior, nasal and overall (mean). A RNFL thickness graph includes the colored normative database range.

Eyes were placed into 1 of 3 categories that indicate comparison versus normative database. Green: within normal limits, with values inside the 95% normal range. Yellow: borderline, with values outside 95% but within 99% confidence interval of normal distribution ($0.01 < p < 0.05$). Red: outside normal limits, with values outside 99% confidence interval of normal distribution.

STATISTICAL ANALYSIS

Intraclass correlation coefficient (ICC), coefficient of variation (COV), and test-retest variability (TRV) were analyzed to determine the reproducibility of SDOCT with regards to RNFL thickness measurements.

Number of participants were calculated for the 95% lower confidence interval (CI) of ICC 0.8 not to be lower than 0.75, a generally accepted lower value of good reproducibility. The average of ICC, COV and TRV were calculated for the RNFL thickness in each quadrant and overall.

Coefficient of variation was calculated using the standard deviation divided by the mean thickness, expressed as a percentage. The test-retest variability in RNFL thickness, measured in micrometers, was calculated as two times the SD of the two repeated measurements for each measure of RNFL thickness.

RESULTS

One hundred fifty one subjects were enrolled in this study, 79 without glaucoma and 72 with moderate to advanced glaucoma. Mean age was 58 (11) years old (range, 40-86) and 68 (10) years old (range, 44-97) for normal and glaucoma groups, respectively. Table 1 shows demographic characteristics of the study population.

Table 2 summarizes the mean RNFL thickness in all quadrants and overall for normal and glaucomatous groups. The inferior and superior quadrant were the thickest in the normal group and glaucomatous group, respectively. The nasal quadrant was the thinnest in both groups.

RNFL measurements in all quadrants and overall were statistically different comparing normal and glaucomatous eyes, with $p \leq 0.001$.

ICC, COV and TRV for both groups are presented in tables 3 and 4, respectively. The lowest ICC was 0.800 in the nasal quadrant for the normal group and 0.827 in the temporal quadrant for the glaucomatous group. The highest ICC was 0.944 for the mean RNFL in the normal group and 0.964 for the superior quadrant in the glaucomatous group. Even the lower 95 % CIs were greater 0.70, indicating excellent reproducibility of all measurements. The overall RNFL had the lowest COV and the nasal quadrant the highest COV in both groups. TRV showed the lowest for the overall RNFL thickness, 4,85 µm in the normal group and 6.61 µm in the glaucomatous group. The highest TRV was observed in the nasal quadrant for the normal group (11.51 µm) and in the inferior quadrant for the glaucomatous group (14.24 µm). RNFL measurements in glaucomatous eyes were more variable than normal eyes.

Table 1: Demographic characteristics

| Groups | Number | Gender | | Age |
		Male	Female	
Normal group	79	49.37%	50.63%	58 (range 40-86)
Glaucomatous group	72	31.94%	68.06 %	68 (range 44-97)

Table 2. RFNL Thickness in normal and glaucomatous groups

| | Normal group | | Glaucomatous group | |
	Thickness (µm)	Std deviation (µm)	Thickness (µm)	Std deviation (µm)
Mean	96.25	10.28	73.14	15.37
Temporal	71.55	11.69	62.25	12.63
Superior	117.87	14.48	87.02	25.06
Nasal	69.60	12.18	56.45	14.44
Inferior	125.19	15.01	86.86	25.63

Std= standard

Table 3. Intraclass correlation coefficient, coefficient of variation and test-retest variability in normal eyes

	ICC	COV (%)	TRV (µm)
Mean	0.944 (0.921)	2.56	4.85
Temporal	0.895 (0.852)	5.93	8.41
Superior	0.928 (0.898)	4.09	9.49
Nasal	0.800 (0.726)	8.74	11.51
Inferior	0.931 (0.902)	3.80	9.33

ICC= intraclass correlation coefficient, with lower 95% CI (confidence interval) in parentheses; COV= coefficient of variation; TRV= test-retest variability

Table 4. Intraclass correlation coefficient, coefficient of variation and test-retest variability in glaucomatous eyes

	ICC	COV (%)	TRV (µm)
Mean	0.937 (0.908)	4.65	6.61
Temporal	0.827 (0.757)	8.44	9.95
Superior	0.964 (0.947)	7.23	11.15
Nasal	0.877 (0.824)	11.44	11.78
Inferior	0.855 (0.795)	8.60	14.24

ICC= intraclass correlation coefficient, with lower 95% CI (confidence interval) in parentheses; COV= coefficient of variation; TRV= test-retest variability

DISCUSSION

In glaucoma patients the OCT is an important instrument for diagnosis and to follow the progression of the disease. Assessing the reproducibility of the RNFL thickness using OCT is of paramount importance for its use in clinical practice of glaucoma.

This study showed excellent reproducibility of RNFL thickness measurements with Spectralis® in patients without and with moderate to advanced glaucoma (generally defined as 0.75 - 1.00). The lowest ICCs were observed in the nasal quadrant for the patients without glaucoma (0.800) and in the temporal quadrant (0.827) for patients with glaucoma. Even the lower 95% CIs were greater 0.70, indicating excellent reproducibility of all measurements.

Comparing two different SD OCTs (Cirrus™ and Spectralis®), excellent repeatability of RNFL thickness measurement was observed in normal participants for both devices. Using Spectralis® in undilated pupils the average of TRV was 4.95, COV 1.7% and ICC 0.971[11]. In our study the TRV was 4.85, COV 2.56% and ICC 0.944. These differences can be explained because the number and the age of the participants in our study were higher.

Many other studies reported the reproducibility of RNFL thickness using different kinds of OCT. Blumenthal et al., in 1999 studied 10 eyes with glaucoma and 10 normal eyes and reported, using the commercially available OCT, that glaucoma patients were found to be significantly more variable than normal subjects (*P*=0.03). The coefficient of variation for the mean RNFL thickness was significantly smaller (*P*=0.02) in normal eyes (6.9%) than in glaucomatous eyes (11.8%). The coefficient of variation was larger in the temporal and nasal quadrants than in the superior and inferior quadrants, as found in our study[12] .

Studies with Stratus™ OCT showed good RNFL thickness reproducibility. Peripapillary RNFL thickness of 51 subjects with glaucoma was measured using the Standard and Fast scan protocols of Stratus™ OCT 3 times on the same day to determine intrasession variability and on 5 different days within a 2-month period to determine intersession variability. It was observed the RNFL thickness test-retest variability intrasession ranged between 5.2 µm and 17.1 µm (mean 5.2 µm) using standard protocol and between 5 µm and 16.7 µm (mean 5.0 µm) for fast protocol, for clock hours. The test-retest variability of the temporal quadrant and of most temporal clock hours seemed to be less than that of other quadrants or locations, and the Fast scanning protocol, ICC and COV tended also to be worst for the nasal quadrant, similar to our results. The best ICC was 0.98 for both protocols. Intraclass correlation coefficients were essentially all excellent with the mean Standard RNFL and Fast RNFL values having the highest of 0.98[13].

Still assessing the reproducibility of the Stratus™, Budenz et al., performed 3 peripapillary circulars scans in 147 subjects normal and with glaucoma. The ICC was excellent for both Standard (mean 0.97) and Fast (mean 0.95) RNFL measurements. TRV of the quadrants measurements ranged from 3.5 µm to 13.0 µm in normal subjects and 5.2 µm to 13.8 µm in glaucomatous eyes analyzing standard and fast protocols, and four quadrants[14]. Reproducibility of Stratus™ OCT as also studied in 10 normal subjects that were scanned three consecutive times with each of the following: macular scans, RNFL scans, and ONH scans before dilation and three additional times for each scan type after dilation. Similarly, all the subjects were scanned on two additional days within 5 months and the inter and intravisit reproducibility was calculated. The ICCs for the RNFL quadrants were higher after dilation, ranging between 71% and 84%, with the exception of the superior quadrant (ICC was 79% before and 75% after dilation). Stratus™ OCT demonstrated reproducible measurements for NFL thickness, macular thickness, and optic nerve head parameters[15].

The reproducibility of macula thickness using spectral domain OCT (Spectralis®) was studied in 41 normal eyes. Intravisit reproducibility was analyzed by performing 3 scans in the macula. It was

observed excellent reproducibility of retinal thickness measurements with mean difference among measurements of about 1 μm[6].

Other SDOCT were also analyzed as RTVue which the RNFL thickness reproducibility showed the ICC ranged between 0.91 and 0.97 for the healthy group (60 eyes) and 0.86 and 0.97 for the glaucomatous group (76 eyes)[7]. Using the SD SLO/OCT OTI the lowest ICC was 0.961 at 3 o'clock in the normal group (98 eyes) and 0.951 at 4 o'clock in the glaucoma group (79 eyes)[9].

Bendschneider et al., analyzed the RNFL thickness in 170 healthy patients using the SDOCT Spectralis® and observed that the RNFL thickness was significantly associated with age (1.9 μm decline in mean total RNFL thickness per age decade), axial length (total RNFL decrease of -4.79 mm per every increasing 1-mm-axial length) and optic disc area (with a total RNFL increase of 6.28 mm for every 1-mm²-increase in disc area). The total RNFL thickness in the study population was 97.2 ± 9.7 μm, which is comparable to our study (96.25 μm ± 10.28)[10].

Recently, Wu et al., reported the reproducibility of Spectralis in 45 normal patients and 33 glaucoma patients. Their ICCs ranged from 0.977 (temporal) to 0.990 (global and inferior-nasal sector) in normal eyes, and from 0.983 (temporal) to 0.997 (inferior quadrant) in glaucomatous eyes. CVs ranged from 1.45% (overall global) to 2.59% (temporal quadrant) for normal participants and ranged from 1.74% (overall global) to 3.22% (temporal quadrant) for glaucoma patients[16]. There were some differences between this study and the present study: they analyzed the RNFL thickness using more than four quadrants (overall global - 360 degrees), for 4 quadrants (superior, inferior, nasal, and temporal), and then for 4 additional sectors: superior-temporal (TS, 45 to 90 degrees), superior-nasal (NS, 90 to 135 degrees), inferior-nasal (NI, 225 to 270 degrees), and inferior--temporal (TI, 270 to 315 degrees); the number of participants was smaller than ours; the subjects had different types of glaucoma, including pseudoexfoliation glaucoma; and they used the TruTrack image alignment software (i.e., the eye tracking system). Probably because of all these facts theirs ICC and COV were better than the present study, especially because of the tracking system.

Evaluating the impact of Spectralis® self-acting eye tracking system and retest software on the reproducibility of RNFL thickness measurements in glaucomatous and healthy eyes it was observed that the reproducibility can be improved by using the eye tracker, and this gain was significantly higher in glaucomatous than in healthy eyes. In healthy subjects, COVs for RNFL thickness measurements without using the system ranged from 3.5% to 7.4% and with the eye tracker and retest protocol ranged from 1.0% to 2.5%. In glaucoma patients measurements without the eye tracker ranged from 5.8% to 10.5% and with the system ranged from 1.6% to 3.8%. The improvement in reproducibility was significantly higher in glaucomatous than in healthy eyes[17]. Our study showed COV ranged from 4.65% to 11.44% in the glaucomatous group similar to the previous study without using the eye tracker system. Despite not using the eye tracking system, our study showed an excellent reproducibility of RNFL thickness in normal and glaucomatous eyes. It is necessary to emphasize that the patients included in the present study had moderate to advanced glaucoma with difficult to fixation on one or both eyes and it may have contributed to the high COV.

CONCLUSION

The Spectralis® spectral domain OCT equipped showed excellent reproducibility with regards to RNFL thickness measurements in normal and in moderate to advanced glaucoma patients.

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