• Brief Report •

Enhanced depth OCT imaging of the lamina cribrosa for 24 hours

Pedro Naranjo Bonilla[1], Rafael Giménez Gómez[1], David Ríos Jiménez[1], María Luísa Varas Fabra[1], María del Carmen Muñoz Villanueva[2], Rocío García Catalán[1], Pilar Font Ugalde[3], María Soledad Poblador Fernández[4], José Luís Lancho Alonso[4], José María Gallardo Galera[1]

[1]Department of Ophthalmology, Reina Sofía University Hospital, Córdoba 14004, Spain
[2]Methodological Support to Research, Maimonides Institute for Biomedical Research, Reina Sofía University Hospital, Córdoba 14004, Spain
[3]Department of Medicine UCO, Maimonides Institute for Biomedical Research, Reina Sofía University Hospital, Córdoba 14004, Spain
[4]Department of Morphological Sciences, Faculty of Medicine, Cordoba 14004, Spain
Correspondence to: Pedro Naranjo Bonilla. Department of Ophthalmology, Reina Sofia University Hospital, Third Floor, Menéndez Pidal Avenue, Córdoba 14004, Spain. pedro.naranjo.bonilla@gmail.com
Received: 2016-01-04 Accepted: 2016-03-09

Abstract

• The lamina cribrosa thickness (LCT) could be affected by dynamic changes in its structure. Using spectral-domain-optical coherence tomography (SD-OCT), we have studied the behaviour of the laminar region in 14 young subjects over 24h. Significant changes in LCT were observed, depending on the time at which the measurement was taken, with the maximum thickness being observed at 7.30 p.m., and the minimum at 7.30 a.m. This finding could suggests a circadian pattern in the LCT thickness in healthy subjects, which could have implications for the classification, diagnosis and prognosis of both normal and glaucomatous subjects.

• **KEYWORDS:** lamina cribrosa; laminar region; glaucoma; optical coherence tomography; twenty-four-hours

DOI:10.18240/ijo.2017.02.20

Naranjo-Bonilla P, Giménez-Gómez R, Ríos-Jiménez D, Varas-Fabra ML, Muñoz-Villanueva MC, García-Catalán R, Font-Ugalde P, Poblador-Fernández MS, Lancho-Alonso JL, Gallardo-Galera JM. Enhanced depth OCT imaging of the lamina cribrosa for 24 hours. *Int J Ophthalmol* 2017;10(2):306-309

INTRODUCTION

In recent years, the technology of spectral-domain optical coherence tomography (SD-OCT) has allowed researchers to analyze the deeper optic nerve head (ONH) structures, giving rise to a better understanding of the laminar region[1-2]. The lamina cribrosa thickness (LCT) seems to correlate with the higher or lower risk of developing glaucomatous neuropathy[3-6].

The purpose of this study is to analyze the performance of different parameters in the laminar region, such as LCT, lamina cribrosa depth (LCD), prelaminar tissue thickness (PTT) and Bruch's membrane opening (BMO) over a period of 24h in healthy young subjects.

SUBJECTS AND METHODS

The study used an observational design and enrolled healthy volunteers. Participants were recruited from the Medicine University of Córdoba, Spain. The study was approved by Hospital Reina Sofia Review Board. All participants provided written informed consent according to the Declaration of Helsinki.

Inclusion criteria were 1) best-corrected visual acuity ≤0.2 (20/30) logarithm minimum angle of resolution in the study eye; 2) spherical equivalent (SE) within ±6.0 diopters, and cylinder correction within ±3.0 diopters; 3) angle grade 4 in Van Herick angle depth estimation.

Exclusion criteria were 1) either good-quality image (*i.e.* quality score 15) could not be obtained or the borders of the lamina cribrosa (LC) could not be appreciated correctly; 2) abnormal peripapillary retinal nerve fiber layer (RNFL) thickness measurements; 3) abnormal ONH cup-to-disc ratio, glaucomatous optic disc, drusen, anterior ischemic neuropathy, retinal or neurological diseases; 4) smoking.

The subjects were assessed at 4 different times (7:30 p.m., 1:30 a.m., 7:30 a.m. and 1:30 p.m.).

Image Acquisition Protocol The optic disc was evaluated by means of the use of Spectralis SD-OCT (Software 1.7.0.0Heidelberg Engineering, Heidelberg, Germany). and the enhanced depth imaging (EDI) technique. Principles of the EDI technique were thoroughly explained by Park *et al*[7]. The BMO was defined as the distance between both limits of

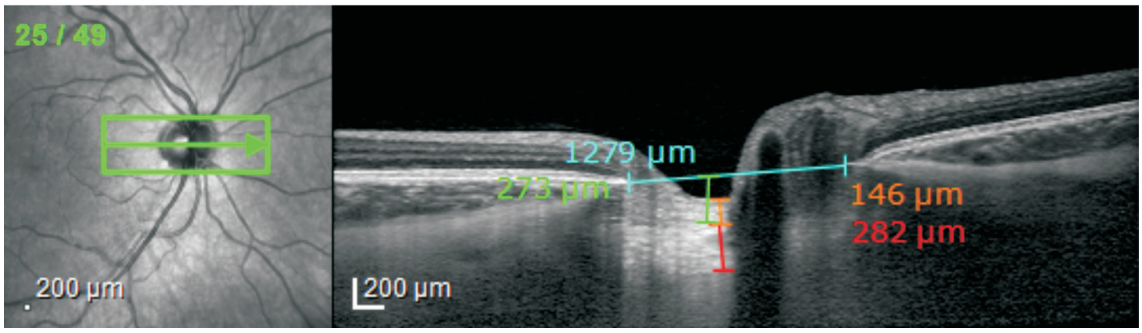

Figure 1 SD-OCT images of the laminar region in a 21-year-old woman The image is assessed by "pixel" option, Bruch´s membrane opening (BMO) is measured by the blue line, LCD is measured by the green line, PTT is measured by the orange line, LCT is measured by the red line. Every measurement was made by the caliper function of the Spectralis SD-OCT.

Bruch's membrane (Figure 1). The LCT was defined as the distance between the anterior border and the posterior border of the LC.

The borders of the LC were considered to be where the highly reflective region started and finished just beneath the retinal nerve fiber layer in the papillary concavity. The LCD was defined as the distance between the anterior border of the LC and the imaginary BMO line. The PTT was defined as the distance between the anterior border of the LC and the anterior face of the prelaminar tissue of the retinal nerve fiber layer. All the measurements were taken by the same ophthalmologist. The observer was blind to the clinical parameters and the timing of the examinations (time 1, time 2, time 3 or time 4) for each scan when assessing their different structures.

At every time of the study two SD-OCT images were taken of every subject. To evaluate intra-observer reproducibility.

Statistical Analysis Statistical analyses were performed using G-STAT version 2.0 (Dpt. of Biometry, GlaxoSmithKline S.A., Madrid, Spain). All data are presented as mean±standard deviation (SD) and median±interquartile range. Given the small sample size, the nonparametric test was used. Statistical comparison for each variable between the times was done using Friedman's test. If Friedman's test yielded a significant result, this was followed by a Wilcoxon test. Spearman correlation analyses were performed to adjust the changes found in the ONH to other factors such as intraocular pressure (IOP), age, SE, central cornea thickness (CCT) and axial length (AL). The $P<0.05$ was considered significant.

RESULTS

From the 21 participants initially enrolled, 7 had to be excluded. Six participants were excluded owing to not completing the 4 measurement times. One participant was excluded owing to poor quality image. We enrolled 14 eyes from 14 patients (42.8% men) (Table 1). The anterior and posterior surface of the LC was seen correctly in the 14 eyes enrolled in the study. Using Anova's test, one factor block design ICC was obtained, the ICC for LCT, LCD, PTT and BMO were 0.94 (0.88-0.97), 0.95 (0.90-0.99), 0.98 (0.95-0.99) and 0.92 (0.82-0.96) respectively. IOP, LCT and PTT variables are simultaneously depicted in Figure 2.

Table 1 Baseline characteristics of the participants in the study

n=14

Parameters	$\bar{x} \pm s$	Median (IR)
Age (a)	19.8±0.9	19.5 (1)
SE (diopters)	-1.65±1.96	-1.15 (3)
CCT (µm)	533.3±28.95	530 (49)
AL (mm)	23.4±0.9	23.6 (0.7)

IR: Interquartile range; SE: Spherical equivalent; CCT: Central corneal thickness; AL: Axial length.

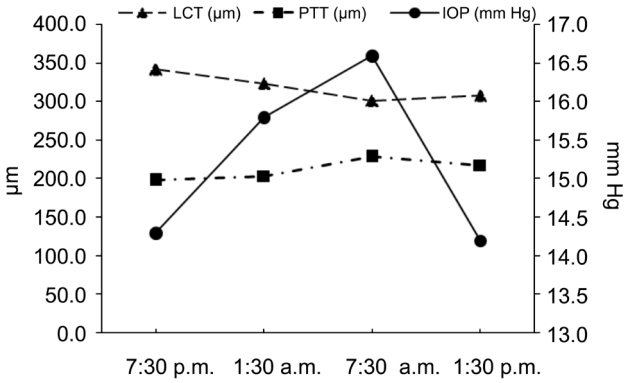

Figure 2 A comparison of 24-hour IOP, LCT and PTT.

We observed significant differences between the times of measuring of IOP ($P<0.05$) and LCT ($P<0.05$) (Table 2).

Using Spearman correlation analysis we found a statistically significant negative correlation between IOP and PTT at 7:30 p.m. (rho=-0.57, $P=0.03$) and at 7:30 a.m. (rho=-0.60, $P=0.02$); we were able to appreciate a negative correlation, not statistically significant, between IOP and PTT at 1:30 a.m. (rho=-0.53, $P=0.05$) and at 1:30 p.m. (rho=-0.22, $P=0.44$).

LCT showed a weak positive correlation with IOP but these changes were not statistically significant at any time (at 7:30 p.m., rho=0.45, $P=0.11$; at 1:30 a.m., rho=0.49, $P=0.07$; at 7:30 a.m., rho=0.21, $P=0.47$ and at 1:30 p.m., rho=0.10, $P=0.71$).

LCD showed a positive correlation with IOP but these changes were not statistically significant at any time (at 7:30 p.m., rho=0.22, $P=0.44$; at 1:30 a.m., rho=0.25, $P=0.39$; at 7:30 a.m., rho=0.47, $P=0.09$ and at 1:30 p.m., rho=0.11, $P=0.71$).

Table 2 Measurements of IOP, LCT, LCD, PTT and BMO at each time of the study

Parameters	7:30 p.m.	1:30 a.m.	7:30 a.m.	1:30 p.m.	*P*
IOP (mm Hg)					
$\bar{x} \pm s$	14.3±1.7	15.8±1.7[a]	16.6±1.7[a,b]	14.2±2.5[b,c]	
Median±IR	14±3	16±2[a]	17±2[a,b]	14.5±4[b,c]	[1]<0.001
LCT (μm)					
$\bar{x} \pm s$	341.9±91.2	323.8±76.1	301.7±83.4[a]	308±56.9	
Median±IR	346±138	327.5±143	307±99[a]	311±91	[1]0.0442
LCD (μm)					
$\bar{x} \pm s$	309.2±79.1	316.1±79.4	311.5±86	309.8±85.4	
Median±IR	275.5±121	306±93	303.5±112	286±117	[1]0.934
PTT (μm)					
$\bar{x} \pm s$	198.3±131.2	203.5±132.3	229±146.2	217.9±127.2	
Median±IR	191.5±161	194±246	215±201	211.5±201	[1]0.526
BMO (μm)					
$\bar{x} \pm s$	1470±146.7	1496.3±150.9	1466.1±154.6	1491.6±160.4	
Median±IR	1447±225	1446.5±286	1429.5±219	1496±211	[1]0.054

IOP: Intraocular pressure; LCT: Lamina cribrosa thickness; LCD: Lamina cribrosa depth; PTT: Prelaminar tissue thickness; BMO: Bruch's membrane opening; SD: Standard deviation; IR: Interquartile range. [1]Comparison between 4 groups by Friedman's test; [a]Significantly different compared with time 1 (7:30 p.m.) by Wilcoxon test; [b]Significantly different compared with time 2 (1:30 a.m.) by Wilcoxon test; [c]Significantly different compared with time 3 (7:30 a.m.) by Wilcoxon test.

No significant correlation was found between the structural changes in ONH and age, SE, CCT or AL.

DISCUSSION

This study has observed "*in vivo*" laminar region performance of the ONH using EDI SD-OCT. The principal finding of this research was the change of the laminar region during the day: first, LCT significantly increased during the diurnal period and decreased during the nocturnal period. Second, the PTT increased during the nocturnal period and decreased during the diurnal period, but these differences were not statistically significant. Third, the LCD did not change during the day. Fourth, the BMO was similar between nocturnal and diurnal periods. According to Fleiss[8] the parameters of the laminar region included in our study had an excellent ICC (≥0.75).

The LC is a dynamic structure. Reis *et al*[9] and Barrancos *et al*[10] have shown anterior displacement of the LC and thickening of the prelaminar tissue along with IOP lowering after glaucoma surgery. These changes are more likely to be observed in young people, and this shows more dynamism and change capacity in the laminar region of young people[11].

We have observed differences in the values of LCT between the different times of evaluation. These changes of LCT coincide with IOP variations. The biggest difference of LCT was found between the 7:30 p.m. measurement and the 7:30 a.m. measurement; this difference was statistically significant. However, we have observed the biggest difference of IOP at 7:30 a.m. and at 1:30 p.m. How could this be explained?

The IOP value at 7:30 p.m. was very similar to the IOP value at 1:30 p.m., but the LCT value at 7:30 p.m. and at 1:30 p.m. was different. Time 4 (1:30 p.m.) was of course preceded by time 3 (7:30 a.m.). The highest IOP value was observed at this time (7.30 a.m.). This considerable pressure could be transmitted to the LC, thereby compressing this structure. Therefore, in spite of the fact that at 1:30 p.m. we have observed the lowest IOP value, the LC might need some amount of time to adapt to the new conditions and expand again[12].

We have observed PTT variations at different examination times, but these differences were not statistically significant. Barrancos *et al*[10] did not find any linear correlation between IOP and PTT, although PTT increased after glaucoma surgery, when IOP drops. Post-surgery edema or vascular changes could explain this increase.

Our results show an inverse correlation between PTT and IOP at the four times of measurement, which was statistically significant at time 1 and at time 3. This leads us to expect a decrease in the PTT when the IOP increases and compresses the tissue. However, the maximum IOP level was observed at time 3 (7:30 a.m.) when the PTT was also highest. Thickening of prelaminar tissue seems to depend on the amount of blood in the nerve fiber layer[13]. Usui *et al*[14] have documented a circadian vascular pattern in the subfoveal choroidal thickness in healthy subjects. In 32 of 38 subjects the maximum subfoveal choroidal thickness was observed during the night, between 3 a.m. and 9 a.m. This interval time coincides with our measurement time 3. We did in fact observe the maximum PTT at 7:30 a.m.; at this time according to the results of Usui *el al*[14] the choroid receives the greatest amount of blood. This result suggests that the PTT could depend on nerve fiber layer vascularization.

Reis *et al*[9] and Barrancos *et al*[10] documented an anterior displacement of the LC when the IOP changed. We did not observe differences in LCD between visits. In our study the

greatest difference between the maximum and the minimum level of IOP recorded was 2.4 mm Hg, in contrast with the work of Reis *et al*[9] and Barrancos *et al*[10] in which the difference was 7 mm Hg and 6 mm Hg respectively.

This study has some limitations. First, only 14 subjects completed the study. In spite of the small sample, some results were statistically significant. A larger and heterogeneous sample would be desirable to validate the results of the study. Second, we only analyzed the central frame in the laminar region of the ONH. Histological and "*in vivo*" studies have demonstrated that the LCT is uniform in the whole LC, and found no differences between the central region and the periphery region of the ONH[4,7,15-16].

Third, young subjects have a thicker PTT which could induce noise in the process of acquisition of images of the LC. However, they have a more transparent cornea, crystalline lens and vitreous humour. In one subject of 21 initially selected for the study (4.7%) it was impossible to mark out the posterior face of the LC. Park *et al*[7] showed a similar result; in 7.3% of the subjects included in his study it was not possible to recognize the posterior border of the LC.

Park *et al*[7] demonstrated that LCT is thinner in normotensional glaucoma (NTG) than in normal eyes or those with primary open angle glaucoma (POAG). Thus, that might explain the glaucomatous damage in NTG eyes with apparently normal IOP levels.

The measurement of LCT might be useful in the early diagnosis of glaucoma and it would be even more relevant if LCT could be used as a screening tool to diagnose subjects with risk of developing NTG. Normally it is difficult to establish NTG in the first stage of the disease, without visual field defects. It is therefore highly relevant to ascertain if LCT alters during the day. Several measurements taken at different times of day are required in order to carry out a diagnosis of a suspected case of glaucoma. In order to complete the study, future investigations of glaucomatous and older subjects would be required, to ascertain if similar behaviour is observable in the laminar region parameters to that noted in younger people.

In conclusion, we successfully obtained images of the ONH structures, in the laminar region during 24h. To the best of our knowledge, this is the first "*in vivo*" study of the laminar region using EDI SD-OCT during 24h, on young and healthy subjects. Our data showed significant changes in the laminar region depending on the measurement time. This study provides an initial approach to understanding the changes in the ONH structures "*in vivo*" in young and healthy subjects depending on the time of measurement.

ACKNOWLEDGEMENTS

Conflicts of Interest: Naranjo-Bonilla P, None; **Giménez-Gómez R,** None; **Ríos- Jiménez D,** None; **Varas-Fabra ML,** None; **Muñoz-Villanueva MC,** None; **García-Catalán R,** None; **Font-Ugalde P,** None; **Poblador-Fernández MS,** None; **Lancho-Alonso JL,** None; **Gallardo-Galera JM,** None.

REFERENCES

1 Inoue R, Hangai M, Kotera Y, Nakanishi H, Mori S, Morishita S, Yoshimura N. Three-dimensional high-speed optical coherence tomography imaging of lamina cribrosa in glaucoma. *Ophthalmology* 2009; 116(2):214-222.

2 Strouthidis NG, Grimm J, Williams GA, Cull GA, Wilson DJ, Burgoyne CF. A comparison of optic nerve head morphology viewed by spectral domain optical coherence tomography and by serial histology. *Invest Ophthalmol Vis Sci* 2010;51(3):1464-1474.

3 Dandona L, Quigley HA, Brown AE, Enger C. Quantitative regional structure of the normal human lamina cribrosa. A racial comparison. *Arch Ophthalmol* 1990;108(3):393-398.

4 Jonas JB, Berenshtein E, Holbach L. Lamina cribrosa thickness and spatial relationships between intraocular space and cerebrospinal fluid space in highly myopic eyes. *Invest Ophthalmol Vis Sci* 2004;45(8): 2660-2665.

5 Jonas JB, Mardin CY, Schlotzer-Schrehardt U, Naumann GO. Morphometry of the human lamina cribrosa surface. *Invest Ophthalmol Vis Sci* 1991;32(2):401-405.

6 Radius RL, Gonzales M. Anatomy of the lamina cribrosa in human eyes. *Arch Ophthalmol* 1981;99(12):2159-2162.

7 Park HY, Jeon SH, Park CK. Enhanced depth imaging detects lamina cribrosa thickness differences in normal tension glaucoma and primary open-angle glaucoma. *Ophthalmology* 2012;119(1):10-20.

8 Fleiss JL. *The design and analysis of clinical experiments*. Wiley Series in Probability and Mathematical Statistics: Applied Probability and Statistics. New York 1986:1-32.

9 Reis AS, O'Leary N, Stanfield MJ, Shuba LM, Nicolela MT, Chauhan BC. Laminar displacement and prelaminar tissue thickness change after glaucoma surgery imaged with optical coherence tomography. *Invest Ophthalmol Vis Sci* 2012;53(9):5819-5826.

10 Barrancos C, Rebolleda G, Oblanca N, Cabarga C, Munoz-Negrete FJ. Changes in lamina cribrosa and prelaminar tissue after deep sclerectomy. *Eye (Lond)* 2014;28(1):58-65.

11 Meirelles SH, Mathias CR, Bloise RR, Stohler NS, Liporaci SD, Frota AC, Simões CC. Evaluation of the factors associated with the reversal of the disc cupping after surgical treatment of childhood glaucoma. *J Glaucoma* 2008;17(6):470-473.

12 Ethier CR, Johnson M, Ruberti J. Ocular biomechanics and biotransport. *Annu Rev Biomed Eng* 2004;6:249-273.

13 Trible JR, Sergott RC, Spaeth GL, Wilson RP, Katz LJ, Moster MR, Schmidt CM. Trabeculectomy is associated with retrobulbar hemodynamic changes. A color Doppler analysis. *Ophthalmology* 1994; 101(2):340-351.

14 Usui S, Ikuno Y, Akiba M, Maruko I, Sekiryu T, Nishida K, Iida T. Circadian changes in subfoveal choroidal thickness and the relationship with circulatory factors in healthy subjects. *Invest Ophthalmol Vis Sci* 2012;53(4):2300-2307.

15 Ren R, Wang N, Li B, Li L, Gao F, Xu X, Jonas JB. Lamina cribrosa and peripapillary sclera histomorphometry in normal and advanced glaucomatous Chinese eyes with various axial length. *Invest Ophthalmol Vis Sci* 2009;50(5):2175-2184.

16 Sanfilippo PG, Grimm JL, Flanagan JG, Lathrop KL, Sigal IA. Application of Elliptic Fourier analysis to describe the lamina cribrosa shape with age and intraocular pressure. *Exp Eye Res* 2014;128:1-7.