Original article

Correlating cup-to-disc ratios measured by HRT-III, SD-OCT and the new color imaging Laguna ONhE procedure

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A B S T R A C T

Objective: To examine correlations between cup-to-disc (C/D) ratios determined by the new Laguna ONhE (optic nerve hemoglobin) color imaging procedure, spectral domain optical coherence tomography (OCT), confocal scanning laser tomography using Heidelberg retina tomography (HRT), and examining retinal images.

Methods: C/D ratio measurements were made on 154 eyes of 154 subjects (52 healthy controls, 36 with ocular hypertension and 66 with primary open-angle glaucoma) using the Laguna ONhE, HRT-III (Heidelberg Engineering) and OCT Spectralis (Heidelberg Engineering) instruments and photographs of the optic disc were examined by a blinded observer (experienced glaucoma specialist).

Results: Global intraclass correlation coefficients (ICC) were: 0.379 (95% CI: 0.233–0.508) for Laguna ONhE-HRT, 0.621 (95% CI: 0.513–0.709) for Laguna ONhE-OCT, and 0.558 (95% CI: 0.398–0.678) for the Laguna ONhE-observer, indicating significant agreement in each case (p < 0.001). The highest ICC was recorded for OCT-observer (0.715; 95% CI: 0.605–0.795).

Conclusions: C/D ratios measured using the Laguna ONhE procedure correlated well with OCT measurements and retinography measurements made by an experienced observer. Best correlation was observed for OCT versus observer measurements. Agreement was good between the Laguna ONhE, OCT and observer measurements, and was somewhat lower between HRT and the remaining procedures.

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Correlación de la relación excavación/papila óptica medida mediante HRT-III, SD-OCT y el dispositivo de colorimetría fotográfica Laguna OnhE

Resumen

Objetivo: Estudiar la correlación existente entre la relación excavación/papila óptica (E/P) medida mediante el nuevo dispositivo de colorimetría fotográfica Laguna OnhE (Optic Nerve Hemoglobin), tomografía de coherencia óptica (OCT) de dominio espectral, tomografía retiniana con láser confocal (HRT) y valoración mediante retinografía.

Métodos: Un total de 154 ojos de 154 sujetos (52 controles, 36 hipertensos oculares y 66 con glaucoma primario de ángulo abierto) fueron estudiados de forma prospectiva determinándose el cociente E/P mediante Laguna OnhE, HRT-III (Heidelberg Engineering), OCT Spectralis (Heidelberg Engineering) y el análisis de fotografías de papilas por parte de un observador experto en glaucoma de forma enmascarada.

Resultados: Los coeficientes de correlación intraclass (CCI) globales fueron: 0,379 (IC 95%: 0,233-0,508) entre Laguna OnhE y HRT; 0,621 (IC 95%: 0,513-0,709) entre Laguna OnhE y OCT; y 0,558 (IC 95%: 0,398-0,678) entre Laguna OnhE y observador, siendo esta concordancia estadísticamente significativa en todos los casos. El mayor CCI se obtuvo entre OCT y observador: 0,715 (IC 95%: 0,605-0,795).

Conclusiones: La medida de E/P estimada por Laguna OnhE presenta buena correlación con la determinada por OCT y la valorada por un especialista en glaucoma. Las mejores correlaciones se encuentran entre OCT y experto. La concordancia fue buena entre Laguna OnhE, OCT y experto, siendo menor con HRT, que presenta una correlación menor con el resto de procedimientos.

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Introduction

The evaluation of the optic nerve papilla (OPN) is essential for diagnosing and managing glaucoma. In addition, the morphological study of OPN constitutes a key element in the early detection of glaucoma, with the determination of the ratio between the papillary cup area and the optic papilla area (C/P) being one of the most widely used parameters. In this context, the analysis of stereoscopic optic papilla photographs carried out by experienced examiners remains as the gold standard within the assessment of optic nerve changes in the development and evolution of glaucoma. However, the clinical description of OPN is subjective as it does not provide quantitative data and could imply high intra- and inter-observer variability. With the development of optic imaging techniques, assessment of optic disc morphology has become more objective and quantitative, leading to widespread use of said techniques in clinical practice.

This study presents a novel method for studying glaucoma based on the color analysis of ocul fundus digital photographs, and the usefulness thereof to estimate the C/P quotient. The objective of this paper is to study the correlation between the C/P ratio, measured by means of this new color photograph device, and that determined by a glaucoma specialist, confocal laser microscopy (HRT) and images taken by spectral domain optic coherence tomography (SD-OCT).

Subjects, material and method

The study included 154 eyes of 154 subjects, in a prospective study: 52 healthy controls, 36 ocular hypertensive subjects (OHT) and 66 with primary open angle glaucoma (POAG): 32 incipient, 19 moderate and 15 advanced.

The inclusion criteria for each group are as follows:

- Controls: intraocular pressure (IOP) <18 mmHg; normal optic papilla and intact neuroretinal ring; normal White-White perimeter
- OHT: IOP ≥ 21 mmHg; 2 normal White-White perimetries
- POAG: reproducible campimetric alterations; pathologic cup and optic nerve appearance. Incipient glaucoma: mean defect (MD) of Octopus TOP G1 White-White perimeter between 2 and 6 dB; moderate glaucoma: MD between 7 and 12 dB; advanced glaucoma: MD ≥ 12 dB.

The following general exclusion criteria were adopted: distance and near corrected visual acuity below 0.5, refraction defect above 3 spherical diopeters and 1.5 astigmatism diopeters, narrow angle in gonioscopic exploration, marked peripapillary atrophy, history of neuro-ophthalmological disease (optic neuritis, multiple sclerosis, etc.), any type of retinopathy or maculopathy, and the type of glaucomatous disease other than POAG, secondary OHT, significant opacities, diabetes mellitus, vascular diseases, neurological diseases, etc.
Examinations were carried out within short periods of time, always in intervals under one week without patient clinical conditions changing within said period. This study was carried out in accordance with the ethical criteria accepted in the Helsinki Declaration and was approved by the Ethics Committee of the San Carlos Clinic Hospital of Madrid.

The C/P quotient was determined by the new color photography device Laguna ONhE (Optic Nerve Hemoglobin, La Laguna, Tenerife, Spain), confocal laser microscopy (HRT-III, Heidelberg Retinal Tomograph, Heidelberg Engineering, Heidelberg, Germany), SD-OCT (Spectralis, Heidelberg Engineering, Heidelberg, Germany) and bidimensional photography analysis of papillae by an observer expert in glaucoma (CMH). Retinographies were captured with the nonmydriatic retinograph (Canon Non-Mydriatic Retinal Camera CR-DGi, Canon Inc., Tokyo, Japan), utilizing specific flash intensities to avoid color saturation.

The Laguna ONhE device automatically locates the papilla in a retinography and shows an expanded image thereof, indicating whether it is a left or right eye. Subsequently, it semi-automatically defines the papilla, allowing the examiner to establish with greater precision the edges thereof. In addition, the device allows the user to establish the vertical and horizontal axes of the papilla as well as its orientation and size, together with a very precise adjustment of the papilla definition. When the definition is accepted as valid, the device automatically generates the results of hemoglobin analysis (HB) in its various presentations (Fig. 1). One of these is the HB concentration slope in the vertical meridian (Fig. 1D). Laguna ONhE offers a C/P quotient value based on an estimation of HB concentrations in the papillary regions of the vertical axis. The mean HB is calculated for several vertical sectors grouped by central, middle of peripheral localization in order to see the distribution from the center toward the periphery. In general, HB diminishes from the center outwardly. The resulting slope has demonstrated to be correlated with the C/P vertical quotient measured with OCT. C/P quotient numbers under 0.64 are established as normal. The amount of HB is assessed with an imaging analysis application equipped with the Matlab image processing tool (The Math Works Inc., Natick, MA, USA) and Java programming language (Oracle Corporation, Redwood Shores, CA, USA).

To establish the C/P quotient, HRT-III automatically calculates a reference plane which is located 50 μm under the mean retinal surface in the area of analysis. On that basis, it establishes the difference between the area corresponding to the papillary cup and the overall disc area. Providing C/P quotient value measured in the vertical meridian. In the case of OCT Spectralis, the measurement was made defining the papillary cup and the optic disc on the basis of ocular fundus photography analysis provided by the study module of the retina nervous fiber layer (RNFL) (Fig. 2). An independent examiner (IRU) determined in all cases the papilla cup by means of this procedure. Based on the ocular fundus photograph and utilizing the high-resolution distance measurement device provided by this platform, the vertical diameters of the cup and the papilla are established in microns (Fig. 3). The ratio between both measures provides the C/P quotient value.

Mean and standard deviation were utilized for describing the characteristics of the subjects included in the study as well as the parameters obtained in the different tests. Mean and standard deviation were taken, or mean and interquartile range in case of asymmetry. The T for Student test was used to analyze the behavior of quantitative variables for each of the diagnostic group categories (in comparisons of one variable with 2 categories) or variance analysis (ANOVA). The significance level was corrected with the Bonferroni test. In all hypothesis comparisons, null hypotheses was rejected with a type I or α error smaller than 0.05. The intraclass correlation coefficient (ICC) was used to determine the match between the various methods applied in the study. The data were analyzed by means of Excel (Microsoft Corporation, Redmond, WA, USA) and SPSS 15.0 for Windows (SPSS, Chicago, IL, USA).

Results

The mean age of the sample was 65.82 ± 12.59 years. The vertical C/P quotient (mean ± SD) was 0.57 ± 0.14; 0.51 ± 0.25; 0.55 ± 0.17; 0.50 ± 0.24, according to Laguna ONhE, HRT-III, OCT Spectralis and the glaucoma expert observer, respectively. The C/P quotient estimated by Laguna ONhE in the studied groups was: 0.51 ± 0.11 in controls; 0.51 ± 0.10 in OHT and 0.65 ± 0.14 in the POAG group, above the values considered as normal by the application.

In the overall population, ICC values were: 0.379 (CI 95%: 0.233–0.508) between Laguna ONhE and HRT; 0.621 (CI 95%: 0.513–0.709) between Laguna ONhE and OCT, and 0.558 (CI 95%: 0.398–0.678) between Laguna ONhE and the observer. This match between Laguna ONhE and the other devices of the study is represented graphically in Fig. 4 by means of Bland–Altman diagrams. The slope value of the regression lines for each case was: Laguna-HRT: −0.75 (CI 95%: −0.58; −0.92); Laguna-OCT: −0.26 (CI 95%: −0.11; −0.41); and Laguna-expert observer: −0.62 (CI 95%: −0.50; −0.75). The match between methods in the overall population of the study is shown in Table 1. The highest ICC of the entire sample was obtained between OCT and observer: 0.715 (CI 95%: 0.605–0.795).

The match between all the methods studied in the various subgroups in which the overall population was divided is shown in Table 2. The highest ICC in the subgroup analysis was obtained between OCT and HRT in advanced glaucoma cases (0.808; CI 95%: 0.530–0.930), followed by the same comparison of devices in the case of OHT (0.733; CI 95%: 0.538–0.854). The match between OCT and observer obtained the third highest ICC, and the case of this analysis was in the control group (0.665; CI 95%: 0.482–0.792). Laguna ONhE obtained its highest match with the expert observer in the incipient glaucoma subgroup (ICC: 0.615; CI 95%: 0.347–0.791).

Discussion

The C/P quotient measure provided by the new Laguna ONhE color photography device is based on optic papilla perfusion and could be useful for diagnosing and following up patients with glaucoma. Retinographies measured the amount of light reflected in various wavelengths. Tissue areas with high content of HB mostly reflect red light, less green and even less blue. According to the assessments carried out by González de
Fig. 1 – Graphic representation of various analyses provided by the Laguna ONHE device. (A) Definition of the optic nerve papilla. When the application opens the retinography, it automatically locates the papilla and shows an expanded image thereof. The examiner establishes the perimeter of the papilla and subsequently the application automatically generates 16 centrally radial sectors, the external limits of which can be adjusted in detail by the examiner. (B) Representation of the papilla in pseudocode or image with color scale at the top. Color code is established based on the Hb percentage existing in each area of the papilla in relation to the established reference value (central retina vessels). The colder colors indicate low amount of Hb (from dark blue to light blue and green), with yellow representing an intermediate value and warm colors such as orange and red up to brown indicating higher presence of Hb, successively. (C) Percentile graph of Hb concentration in each sector with color scale at the top. The papilla is divided in 24 asymmetric sectors arranged as shown in the figure. The 24 sectors are set in groups of 3 (each having the same thickness), forming 45° sectors. As the figure shows, the sectors are finally distributed in 3 concentric rings. (D) Hb concentration slope in the vertical meridian. Mean Hb for several vertical sectors grouped by central, middle of peripheral location is calculated in order to see the distribution thereof from the center to the periphery. The application provides an estimation of the vertical C/P quotient and provides the numeric data taken as normal reference.

| Table 1 – ICC between the different devices throughout the sample (n = 154). |
|---------------------------------|------------------|------------------|------------------|---|
|                                | Laguna ONHE (0.57 ± 0.14) | HRT-III (0.51 ± 0.25) | SD-OCT (0.55 ± 0.17) | p  |
| HRT-III                         | 0.379             |                  |                  | 0.379 < 0.001     |
| SD-OCT                          | 0.621             | 0.644            |                  | 0.621 < 0.001     |
| Expert observer (0.50 ± 0.24)   | 0.558             | 0.649            | 0.715            | 0.558 < 0.001     |
| p < 0.001                       | p < 0.001         | p < 0.001        | p < 0.001        | 0.558 < 0.001     |
Fig. 2 – Graphic representation provided by the OCT Spectralis retina nervous fiber layer (RNFL): ocular fundus photograph, indicating the location of the section; a tomographic section of the retina layers, enhanced in the RNFL; RNFL thickness quantification (in µm) per sector and classification thereof by means of a color map; and representation of the RNFL profile overlapping the color map.

Table 2 – ICC between the different devices in the sample subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Laguna ON3,E</th>
<th>HRT-III</th>
<th>SD-OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRT-III</td>
<td>0.263 (p = 0.028)</td>
<td>0.606 (p &lt; 0.001)</td>
<td>0.665 (p &lt; 0.001)</td>
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<tr>
<td>SD-OCT</td>
<td>0.498 (p &lt; 0.001)</td>
<td>0.600 (p &lt; 0.001)</td>
<td>0.586 (p &lt; 0.001)</td>
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<tr>
<td>Expert observer</td>
<td>0.348 (p = 0.005)</td>
<td>0.600 (p &lt; 0.001)</td>
<td>0.535 (p = 0.001)</td>
</tr>
<tr>
<td>OHT</td>
<td></td>
<td></td>
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<tr>
<td>HRT-III</td>
<td>0.158 (p = 0.173)</td>
<td>0.733 (p &lt; 0.001)</td>
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<tr>
<td>SD-OCT</td>
<td>0.305 (p = 0.032)</td>
<td>0.632 (p &lt; 0.001)</td>
<td>0.535 (p = 0.001)</td>
</tr>
<tr>
<td>Expert observer</td>
<td>0.097 (p = 0.281)</td>
<td>0.308 (p = 0.039)</td>
<td>0.535 (p = 0.001)</td>
</tr>
<tr>
<td>Incipient glaucoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRT-III</td>
<td>0.323 (p = 0.032)</td>
<td>0.308 (p = 0.039)</td>
<td></td>
</tr>
<tr>
<td>SD-OCT</td>
<td>0.479 (p = 0.002)</td>
<td>0.535 (p = 0.001)</td>
<td>0.460 (p = 0.019)</td>
</tr>
<tr>
<td>Expert observer</td>
<td>0.615 (p &lt; 0.001)</td>
<td>0.336 (p = 0.070)</td>
<td>0.554 (p = 0.011)</td>
</tr>
<tr>
<td>Moderate glaucoma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HRT-III</td>
<td>-0.034 (p = 0.556)</td>
<td>0.479 (p = 0.015)</td>
<td></td>
</tr>
<tr>
<td>SD-OCT</td>
<td>0.298 (p = 0.096)</td>
<td>0.336 (p = 0.070)</td>
<td></td>
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<tr>
<td>Expert observer</td>
<td>0.290 (p = 0.103)</td>
<td>0.808 (p = 0.001)</td>
<td></td>
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<tr>
<td>Advanced glaucoma</td>
<td></td>
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<tr>
<td>HRT-III</td>
<td>0.444 (p = 0.038)</td>
<td>0.521 (p = 0.017)</td>
<td></td>
</tr>
<tr>
<td>SD-OCT</td>
<td>0.564 (p &lt; 0.010)</td>
<td>0.808 (p = 0.001)</td>
<td></td>
</tr>
<tr>
<td>Expert observer</td>
<td>0.590 (p = 0.007)</td>
<td>0.521 (p = 0.017)</td>
<td>0.554 (p = 0.011)</td>
</tr>
</tbody>
</table>

* p < 0.05.

la Rosa et al., the lower the amount of Hb the higher will be the reflection of green and blue light accordingly, in areas with atrophy or poor vascularization (papilla cup), the proportion of reflected green and blue light increases, and this is shown in the image as whiter shades (paleness). In the past, similar glaucoma detection computer systems have been published based on ocular fundus photograph analysis which automatically analyzed the C/P quotient, with very good results. The Laguna ON3,E application utilizes automatic segmentation mathematic algorithms which identify the retinal vessels
in the edge of the papilla. Likewise, it provides a C/P quotient value based on estimated Hb concentrations in the papilla regions of the vertical axis. According to the present study, this estimated measure exhibits a good correlation with that established by OCT and by the glaucoma expert. The match between Laguna ONhE, OCT and the glaucoma expert was good in the overall sample (slightly higher with OCT; ICC: 0.621; p < 0.001), and lower with HRT (ICC: 0.379; p < 0.001), which presented a lower correlation with the rest of procedures. In the subgroup analysis a similar result was obtained, with the match between Laguna ONhE and HRT being lower than with other methods in all the subgroups, except for OHT (ICC was lower with the expert observer). Laguna ONhE obtained its highest match with the expert observer in the incipient glaucoma subgroup (ICC: 0.615), exceeding in this group and in the advanced glaucoma group the match with OCT, which here again obtained the highest ICC values in the majority of studied subgroups.

The vertical C/P parameter is an HRT parameter which has demonstrated usefulness in differentiating controls and glaucomas. Some studies in large OHT populations have demonstrated that the measures obtained with HRT exhibit good correlations with the estimates based on stereo photographs of the ratio between the C/P quotient, both horizontally and vertically. In addition, it has been seen that these associations remain after adjustments by age, disc area and IOP. When comparing HRT with SD-OCT (SOCT Copernicus HR; OPTOPOL Technology S.A., Zawiercie, Poland) the highest correlations between both instruments were observed in the cup area (r = 0.783; p = 0.000) and in the C/P quotient (r = 0.669; p = 0.000), whereas the lowest correlations were observed in the papillary area (r = 0.100; p = 0.037), ring area

Fig. 3 – Detailed view of ocular fundus color photograph: enlarged and centered on the optic nerve papilla, showing how the vertical diameters (in µm) of the cup and the papilla were determined by the high-resolution distance measuring function provided by the platform.

Fig. 4 – Bland–Altman graphs showing the match between Laguna ONhE and the other devices included in the study in the overall population. (A) Laguna ONhE – HRT. (B) Laguna ONhE – expert observer. (C) Laguna ONhE – OCT.

(r = 0.275; p = 0.000), backup volume (r = 0.005; p = 0.391) and ring volume (r = 0.021; p = 0.346). A study carried out by Schuman et al. found that the papillary area, the C/P quotient and the papillary cup area were higher with OCT when compared with HRT, one the opposite occurred with the neuroretinal ring volume. In the present study, the C/P quotient with OCT (0.55 ± 0.17) was higher than that obtained with HRT-III
(0.51 ± 0.25), with this difference being the statistically significant (t-test; p = 0.004).

In some studies it was perceived that the measures obtained with OCT were higher than those obtained with HRT-III, with the exception of the mean and maximum cup depth. The value of the correlation coefficients for the papillary area and the C/P quotient were 0.367 and 0.681 respectively, a number which was very similar to those found in the present study (HRT ICC compared with OCT for C/P quotient: 0.644; CI 95%; 0.536–0.730).

The best correlations found in this study correspond to OCT and the expert examiner. The method applied in the present study for assessing the C/P quotient with OCT Spectralis, based on the RNFL study module, possibly exhibits lower precision than the parameters available with other SD-OCT such as Cirrus (Carl Zeiss Meditec, Dublin, Ireland), which provides a C/P quotient directly. However, it is most similar to the papilla analysis carried out by an observer, which could enhanced the high match between these 2 methods as regards their results.

In essence, the criterion which is more useful for an observer to differentiate between the area corresponding to the neuroretinal ring and the papillary cup is the color of each area, which is similar to the analysis carried out by the color photography application. However, additional factors come into play, including the distribution and curve of papillary vessels, etc., the interpretation of which could be more limited for the other color photography application. In the present case, it must be pointed out that the optic papilla analysis with bidimensional photographs could be a limitation of the study in relation to be more precise analysis provided by stereo photographs.

In the present study it has been observed that ICC for the vertical C/P quotient between OCT Spectralis and an expert observer was 0.715 (p < 0.001), which is higher than in the numbers found in some studies which compared to the parameters of the optic disc assessed by means of stereo photography and OCT Cirrus (r = 0.497). The match between OCT and observer obtained the highest ICC within the comparisons of the entire sample and the third highest ICC in the subgroup analysis, in this case in the healthy control subgroup where it is possibly easier for the observer to assess the value of the C/P quotient and match the OCT reading.

The Bland–Altman graphs (Fig. 4) show that, measuring the C/P quotient with OCT, the value tends to be higher in moderate and advanced glaucomas than in controls or subjects with OHT. However, Laguna ONhE systematically tends to provide somewhat higher C/P readings in OHT and controls than OCT and values generally somewhat lower in more pathological groups. A similar result is obtained in the Laguna-HRT and Laguna-observer readings. The negative value of the regression line slope in these graphs explains the performance of Laguna ONhE when comparing it with the other studied diagnostic methods. As commented above, Laguna ONhE produces a C/P quotient value based on estimated Hb concentrations in the papillary regions of the vertical axis. Even though the utilization of these color parameters is somewhat similar to the visual analysis made by the observer (although with the differences described above), the application of morphometric criteria utilized by OCT and HRT is very different and could provide less precise measures of this variable, which could be a possible explanation for these results. At this point, it must be recalled that Laguna ONhE was not primarily designed for measuring the C/P quotient but to obtain an indirect measure through estimates.

In conclusion, the C/P quotient provided by Laguna ONhE exhibits good correlation with the semiautomatic readings based on OCT Spectralis images and those assessed by a glaucoma expert. The best C/P correlations obtained by different procedures are between the vertical C/P OCT reading and that of the expert observer. The correlation between Laguna ONhE, OCT and the expert was good but lower with the HRT-III vertical C/P reading which exhibited a lower correlation with the rest of procedures. Even though the main purpose of Laguna ONhE is not to measure the C/P ratio, the correlation between papillary cup assessment obtained with this procedure and other structural diagnostic tools, as well as that assessed by an expert in glaucoma is high.

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**Conflict of interests**

No conflict of interests has been declared by the authors.

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