Original article

Variability and reproducibility of 3 methods for measuring the thickness of the nerve fiber layer

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ABSTRACT

Objective: To estimate the variability and reproducibility of confocal tomography (HRT), scanning laser polarimetry (GDx) and optical coherence tomography (OCT-Cirrus) to determine the thickness of the layer of ganglion fibers.

Method: A total of 75 normal eyes were examined twice. Inter-individual variability was analyzed after standardizing the results. The coefficient of variation was used to measure the variability between tests, and the Pearson coefficient was used to analyze the correlation between variables.

Results: The inter-individual variability was similar in GDx (8.9%) and OCT (11.1%), but very high in HRT (30.0%). No instrument detected significant changes with age. The coefficient of variation of the total thickness between the examinations of the same subject was significantly lower (p < 0.05) in GDx (1.4) than in OCT (2.0), but very high in HRT (6.4). The same was true when analyzing the upper fibers (GDx = 1.8, OCT = 2.9, HRT = 6.6), but not with the lower ones, where the only significant differences were observed with HRT (GDx = 2.2, OCT = 2.7, HRT = 7.0). Among the results of OCT and GDx, there was a significant correlation when comparing the first (r = 0.46, p < 0.0001) and second examinations (r = 0.52, p < 0.0001). However there was no significant relationship between the data provided by HRT for the two remaining instruments (p > 0.05).

Conclusions: There is a wide variation in the inter-individual and inter-test measurement of the thickness of the nerve fibers layers using HRT. GDx has, in this respect, slight advantages over OCT.

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Variabilidad interindividual y reproducibilidad de tres métodos de medida del espesor de la capa de fibras nerviosas

R E S U M E N

Objetivos: Calcular la variabilidad interindividual y reproducibilidad de la tomografía confocal (HRT), polarimetría láser (GDx) y tomografía de coherencia óptica (OCT Cirrus) para determinar el espesor de la capa de fibras ganglionares.

Método: Se examinaron 2 veces 75 ojos normales. La variabilidad interindividual se analizó previa normalización de los resultados. Para medir la variabilidad entre exámenes se utilizó el coeficiente de variación y para analizar la correlación entre variables, el coeficiente de Pearson.

Resultados: La variabilidad interindividual fue similar en GDx (8,9%) y en OCT (11,1%) pero muy elevada en HRT (30,0%). Ningún instrumento detectó cambios significativos con la edad. El coeficiente de variación del espesor total, entre 2 exámenes del mismo sujeto, fue significativamente inferior (p<0,05) en GDx (1,4) que en OCT (2,0) y muy elevado en HRT (6,4). Lo mismo ocurrió al analizar las fibras superiores (GDx=1,8; OCT=2,9; HT=6,6), pero no las inferiores, donde solo se observaron diferencias significativas con HRT (GDx=2,2; OCT=2,7; HRT=7,0).

Entre los resultados de OCT y GDx existió una correlación significativa al comparar los primeros (r=0,46; p<0,0001) y los segundos exámenes (r=0,52; p<0,0001). Sin embargo, no se observó ninguna correlación significativa entre los datos aportados por HRT respecto a los 2 instrumentos restantes (p>0,05).

Conclusions: HRT presenta un exceso de dispersión interindividual y de variabilidad intertest en la estimación del espesor del haz de fibras nerviosas. GDx presenta, en este aspecto, ligeras ventajas respecto a OCT.

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Introduction

Glaucoma is an optic neuropathy characterized by progressive loss of retinal ganglion cells which produces localized or diffused thinning of the retina nervous fiber layer (RNFL) and visual field alteration.1

Automated perimetry is highly reproducible and an excellent method for measuring functional loss although the structural alteration of the RNFL and the optic nerve head was limited to qualitative analysis.2,3

In recent decades new diagnostic tools have become available that provide quantitative measurements of said structures in an objective manner and less subject to the influence of the examiner on results, thus increasing reproducibility and reliability. Said tools include the confocal Heidelberg Retina Tomograph (HRT), optic coherence tomography (OCT) and laser polarimetry (GDx), which perform an exact structural analysis of the RNFL and the optic nerve head in the search for morphological alterations secondary to the disease.4–7

At the present time, the diagnostic of glaucomatous disease is mainly based on the exploration and evaluation of the optic nerve, the RNFL and the visual field in addition to intraocular pressure measurements, gonioscopy, corneal pachymetry, etc.8

However, for a diagnostic method to be efficient it must be sensitive, specific and reproducible. While sensitivity depends on the ease with which it is altered by the disease, specificity mainly depends on clear definition and low dispersion of normal measurements.

In order to determine said characteristics, the objective of this study was to observe the interindividual distribution and reproducibility of RNFL thickness data as provided by HRT, GDx and OCT-Cirrus in healthy subjects.

Materials and methods

A transversal study including 75 eyes of 75 subjects who underwent a complete ophthalmological exploration that included Spark strategy automated perimetry with the Easy-field perimeter (Oculus, Wetzlar, Germany), HRT II (Heidelberg Retina Tomograph, Heidelberg Engineering, GMBH, Heidelberg, Germany), GDX VCC (Carl Zeiss Meditec Inc., Dublin, Ireland) and OCT-Cirrus (Carl Zeiss, Meditec, Dublin, Ireland). All the examinations were carried out twice, either on the same day or in the next few days.

All the subjects had intraocular pressure (IOP) values of 21 mmHg or less, automated perimetry compatible with the normal values, papillae without signs of glaucomatous optic neuropathy and absence of family history involving glaucoma.

The subjects were selected according to the following inclusion criteria: age comprised between 18 and 18. visual acuity of 0.8 or greater (Snellen scale), refraction defect under 5 sphere dipters and 3 cylinder dipters as well as transparent optic media. The patients who were not included in the study exhibited severe hematological or cardiovascular...
disease, traumatism or surgery history, retinopathy of any etiology, angle anomalies or impossibility of carrying out any of the tests included in the protocol.

The study was executed in accordance with the ethical rules of the 1964 Helsinki Declaration as well as with the prior consent of the subjects who voluntarily accepted their inclusion in the study after being duly informed of its methodology and objectives.

HRT performs 3 series of scans of 32 equidistant tomographic sections. On the basis of these images, the computer program included in the device carries out a 3-D reconstruction of the analyzed structure, which is displayed as an image with topographic values. For the device to provide all the parameters, the operator must define the contour of the papillary area. This gives rise to a limitation and can increase variability. However, the instrument memorizes said contour for each patient and applies it automatically in successive readings, thus minimizing said variability. The data were analyzed according to the Moorfields regression analysis (MRA). This analysis was proposed by Wollstein et al. with a papillary assessment that compared the values of the neuroretinal ring area of an evaluated subject with the normality values included in a database, adjusted for age and pupil size.

In turn, OCT-Cirrus analyzes RNFL and the papilla by means of scans obtaining data on global thickness per quadrant and sector in addition to other morphological parameters of the disk, cup, etc.

Finally, GDx VCC utilizes laser polarimetry and executes a compensation of anterior segment birefringence for each eye and calculates the RNFL thickness in the peripapillary area on the basis of the light reflected in the retina. Calculations are performed on a strip of fixed sized tissue centered on the optic nerve and obtaining morphological parameters comprising upper and lower average, TSNIT deviation and maps of the studied area. In addition it provides an overall index which is higher with increased probability of glaucoma: the nerve fiber indicator (NFI).

Even though some studies had indicated that the NFI index of GDx is the most sensitive parameter of this device for diagnosing glaucoma, its calculation method ponders various parameters and does not directly indicate the nervous fiber layer thickness. Due to being more compatible with the measurements of the other 2 instruments, this study analyzed the area of the TSNIT curve (circular thickness beginning on the temporal side of the papilla and following with the upper, nasal and inferior side to return to the initial temporal side).

In what concerns OCT-Cirrus, as the manufacturer does not provide the option of exporting data of all the sectors where the fiber layer thickness has been measured, the graphs were digitalized with the Engauge application (Free Software Foundation Inc, Boston, MA, USA) following a sequence similar to the above described TSNIT.

The results were analyzed by means of MedCalc 7.3 (MedCalc, Mariakerke, Belgium) and Excel (Microsoft Corporation, Redmond, WA, USA). Interindivudual variability was analyzed after standardizing the results vis-à-vis their mean value. For measuring variability between two examinations the variation coefficients were applied, while Pearson’s correlation coefficient was used to analyze the correlation between variables.

\[ \text{Fig. 1 – Standardized interindividual variability of the 3 procedures.} \]

\[ \text{Results} \]

The series comprised 22 males and 53 females with a mean age of 41.9 years (normalized standard deviation [SD] = 12.4). Interindivudual variability was similar in GDx (normalized standard deviation, SD = 8.9%) and in OCT (SD = 11.1%) but much higher in HRT (SD = 30.0%) (Fig. 1).

None of the instruments detected significant changes with age: HRT \( r = 0.02 \), GDx \( r = 0.03 \) and OCT \( r = -0.20 \) (\( P > 0.05 \) in the 3 cases).

The variation coefficient for total thickness, between 2 examinations of the same subject, was significantly lower (\( P < 0.05 \)) in GDx (average = 1.4; SD = 1.3) than in OCT (average = 2.0; SD = 2.6) and very high in HRT (average = 6.4; SD = 6.9). Similar results were obtained when analyzing the superior fibers (GDx average = 1.8; SD = 1.7; OCT average = 2.9; SD = 4.8; HRT average = 6.6; SD = 6.8), but not the inferior fibers where no significant differences were observed (GDx average = 2.2; SD = 2.0; OCT average = 2.7; SD = 2.1; HRT average = 7.0; SD = 14.3).

A significant correlation was found between the results of OCT and GDx when comparing the initial examinations (\( r = 0.46; P < 0.0001 \)) and also when comparing the second ones (\( r = 0.52; P < 0.0001 \)) (Fig. 2). However, no significant correlation was observed between the data provided by HRT and those provided by the other instruments (\( P > 0.05 \) in all cases).

When the average value of the tool measurements was used it was observed that 95% of the fiber layer thickness of the sample closely matched that shown by the manufacturer graphs (Fig. 3). However, the 5% value was somewhat inferior (7% in average) and 1% substantially inferior (11% in average) against the standards database. The data obtained between sectors 85 and 90 corresponding to the supero-nasal area were particularly different. Here, the 5% value which is generally used as the lower normality limit was 19% lower than that of the standards database.
Discussion

The profitability of the 3 diagnostic instruments of the study was previously validated in other studies, although none was applied to the same healthy population sample to determine the reproducibility and interindividual variability differences. The results of this study demonstrate that interindividual variability of OCT and GDx results is comparable. This is not the case with HRT, which exhibited significant dispersion. Specific and reasonable abnormality criteria are necessary to differentiate normality from disorders, and in this area the data provided by HRT appear to be clearly inferior to those of the other 2 devices. It must be taken into account that the fiber layer thickness measurement made by HRT is indirect and does not seem to be the best index produced by this device.

None of the 3 systems was able to detect with precision the neuronal loss occurring with age. However, OCT reached probability levels that lead us to consider that significant correlations could be achieved by increasing the sample size. In any case, this relationship would be weak and this could question the capacity of the system to execute corrections of the standard database with age, as the software seems to suggest.

On the other hand, HRT exhibits considerably higher variability between examinations when compared to the other 2 systems. This will inevitably reduce its diagnostic and progression detection capabilities.

GDx only appears to be superior in what concerns lower fluctuation between examinations, although this involves mainly the upper fibers group which is infrequently compromised in glaucoma. On the other hand, even though the correlation between the results of GDx and OCT is significant, it cannot be defined as excellent. For this reason, the data provided by both instruments can be seen as related but not comparable. This would explain the moderate-good (albeit not excellent) match between the diagnostic results of both instruments.

Some authors have suggested that the RNFL is the first ocular structure which can evidence damages in glaucomatous patients, proceeding in up to 6 years of the appearance of defects in conventional perimetry. However, other authors consider that the first detectable damage can be functional or structural, according to each particular case.

There is no agreement about which instrument can lead to a better diagnostic. It has been stated that, comparing the performance of OCT and GDx with the Fourier analysis, better results are obtained in TSNIT RNFL readings with OCT, but the results were not statistically significant.

Several papers have concluded that the measurements with the best diagnostic value are the discriminating function of HRT, the mean RNFL thickness of OCT and the nervous fiber index of GDx, although the diagnostic capacities between these devices are not vastly different. However, it must be emphasized that their results are not always in agreement. Therefore, it is necessary to make a joint assessment of the 3 techniques because none of them exhibits sufficient sensitivity and specificity to merit their exclusive use.

Central corneal thickness (CCT) is a significant risk factor for developing glaucoma. This relationship has been suggested because CCT is an indirect indicator of the general structure and biomechanical properties of the eye. However, there is no statistically significant relationship between the RNFL thickness measured with HRT, GDx and OCT and CCT.

In view of the above, it can be concluded that GDx yields lower fluctuation between examinations, above all in the superior fiber group examination. A moderate significant correlation is observed between GDx and OCT as both provide a precise RNFL analysis and produce useful quantitative parameters. HRT provides indirect measurements and exhibits high dispersion and variability between examinations. Accordingly it may present alternative advantages but not specifically RNFL thickness measurements.

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Conflict of interests

No conflict of interests has been declared by the authors.

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