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Abstract

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Reference


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Reliability of Different Image Analysis Methods for Scanning Laser Doppler Flowmetry

ABSTRACT  Purpose: To assess reliability and reproducibility of different analysis methods for retinal capillary flow, volume, and velocity from scanning laser Doppler flowmetry (SLDF) topography. Material and Methods: SLDF topography analysis using the default retinal flowmeter (HRF) were compared to that using automatic full-field perfusion image analyzer (AFFPIA) and quantified as intraclass correlation coefficients (ICC). Results: The AFFPIA full-field method had the highest reliability, with ICC 0.99 for capillary flow. The reproducibility using the AFFPIA full-field method was high ICC 0.74 for capillary flow. Conclusions: The AFFPIA full-field method is highly reliable and superior to the default HRF software.

KEYWORDS  AFFPIA; flowmetry; image analysis; reliability; reproducibility

INTRODUCTION

The retina can be viewed directly and non-invasively, allowing an assessment of the human microvasculature in vivo. Recent studies show that structural retinal microvascular changes, such as the presence of retinopathy or variations in retinal vascular caliber, are predictive of a range of systemic cardiovascular and ocular conditions. Development in scanning laser Doppler flowmetry (SLDF) has made quantitative assessment possible of functional changes in retinal blood flow. This technology allows further understanding of physiological and pathological alterations in the retinal microcirculation in patients with cardiovascular disease, diabetic retinopathy, age-related macular degeneration, and glaucoma.

A commonly used SLDF device is the Heidelberg Retinal Flowmeter (HRF, Heidelberg Engineering, Heidelberg, Germany), which measures retinal capillary flow, volume, and velocity. While the HRF has been applied to studying different ocular diseases, including glaucoma, diabetic retinopathy, and age-related macular degeneration, the reliability of measurement captured on the HRF is uncertain. The HRF captures a topographic image, but these images require a trained observer to analyze and calculate the summary measurements. There are very little data on the “intra-observer reliability” of such analyses (i.e., the ability of a trained observer or grader to obtain consistent
summary measurements when analyzing the same topographic images of the HRF more than once. Most previous research, with varying terminology, assesses reproducibility (i.e., the intra-subject variation of measurements analyzed from topographs taken over time, where this analysis may be undertaken by one or many trained graders).

We aim to assess intra-observer reliability of retinal capillary flow, volume and velocity measurements of the HRF, and intra-subject reproducibility of multiple topographic scans obtained over a short period of time on the same examination day, using four analysis methods: the original default HRF software with cursor options of 10 × 10 pixels (method 1) or 20 × 20 pixels (method 2), and the automatic full-field perfusion image analyzer (AFFPIA) with cursor options of 20 × 20 pixels (method 3) or a full-field option (method 4).

METHODS

We conducted a cross-sectional study of 13 healthy subjects who were volunteers from the administrative and medical staff in the Department of Cardiology at the Austin Health Hospital, University of Melbourne, Victoria, Australia. The Research Ethics Committees of Austin Health, Royal Victorian Eye and Ear Hospital, and the University of Melbourne approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki, and informed written consent was obtained from each participant. Eligibility criteria were normal visual function and no pathological findings upon slit lamp and fundus examinations. All subjects had not eaten or smoked for at least 3 hr and had rested prior to measurement by a single experienced grader.

Scanning Laser Doppler Flowmetry (SLDF)

The technique of SLDF on the HRF has been described in detail elsewhere. Briefly, the examination was performed in the sitting position after a short rest (about 10–15 min) at room temperature and diffuse natural light, with pupil undilated. Measurements were performed in a chosen region within 1.5 to 2 disc diameters temporally from the optic disc margin. An area of 2.56 × 0.64 × 0.30 nm was scanned within 2 sec at a resolution of 256 points × 64 lines × 128 lines with the default 780-nm wavelength laser head installed in the HRF camera.

During the data acquisition, the participant was asked to fixate on a mounted artificial light spot placed approximately 2 m in front of him/her but slightly higher than the level of neutral eye position to prevent blinking. A retinal scanning image was taken from the right eye of each person. Repeated images were taken after 5 min and 10 min.

Image Analysis Methods and Modes

We followed the original HRF Operation Manual 1.02 (Heidelberg Engineering). Image analysis was performed using the original default HRF MSDOS-based analysis software (Figure 1 shows 20 × 20 pixel cursor option) and the AFFPIA (Fig. 2) in accordance with the methods outlined elsewhere. Briefly, AFFPIA calculates the Doppler frequency shift of the hemodynamic flow of each pixel from the entire image, while the standard HRF software only analyzes within a given small-framed area (i.e., the pixel cursor). For a valid estimation of retinal blood flow, AFFPIA, unlike the HRF software, adjusts brightness to mask under- or over-exposed pixels. AFFPIA also eliminates noise from artificial movement (saccades), avoids measuring extremely wide retinal vessels and the optic nerve head, and accounts for the heart phases (systole and diastole) by averaging the differences between the two phases. Figure 2 shows the steps of imaging processing.

Two different analysis options were utilized from each of two different image analysis software programs, resulting in four methods tested:

1. HRF software, 10 × 10 pixel cursor option (the default mode).
2. HRF software, 20 × 20 pixel cursor option (Fig. 1).
3. AFFPIA, 20 × 20 pixel cursor option.
4. AFFPIA, full-field option (Figure 2).

Reliability and Reproducibility

In this study, “intra-observer reliability” refers to comparison of initial and repeated image analysis measurements by one grader (AK), approximately 10 days apart, from the same topographic scanning image. “Intra-subject reproducibility” refers to comparison of measurements from three different scanning topographic images from the same person, taken on the same day 5 min apart (baseline, 5 min later, 10 min later), and analyzed once by the same grader.
Statistical Analysis

All image analysis data were manually exported into a database. Intra-observer reliability and intra-subject reproducibility of multiple topographic images were assessed using intraclass correlation coefficients (ICC) with exact 95% confidence intervals. Reliability utilized per-image data ($n = 39$ images, 3 from each of 13 patients) and data from the first scan per person ($n = 13$ images), while reproducibility utilized all three images from the same person ($n = 13$ patients). Reliability was calculated for all four different analysis modes. Reproducibility was calculated using only the most reliable image analysis mode. All analyses were undertaken using Intercooled Stata 9.0 for Windows (StataCorp, College Station, TX, USA).

RESULTS

The 13 hospital employee volunteers who participated in this study were predominately female (77%).
Caucasian (85%), non-smokers (85%), with education qualifications higher than high school (92%). Average age was 39.7 years (range 24–55), and median BMI was 23.9 (70% normal weight, 15% overweight, and 15% obese). One participant (8%) had treated hypertension. No study participant had a history of diabetes, hypercholesterolemia, coronary heart disease, stroke, glaucoma, age-related macular degeneration, diabetic retinopathy, cataract, or retinal arteriovenous occlusion.

Figure 3 shows the retinal capillary flow as analyzed 10 days apart by the four image analysis methods. The AFFPIA full-field method provided near-identical results (bottom right panel).

Table 1 presents intra-observer reliability for capillary flow, volume, and velocity measured using the four different image analysis methods in 39 scans taken from 13 participants (3 scans from each subject). The AFFPIA full-field method gave the greatest reliability for flow (ICC = 0.99, 95% CI 0.98, 0.99), volume (ICC = 0.99,
TABLE 2 Reproducibility of capillary flow measurements from topographic scans taken at baseline, 5 min, and 10 min later using automatic full-field perfusion image analyzer full-field mode

<table>
<thead>
<tr>
<th>Reproducibility of flow measurements ICC (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>Over 5 min (baseline vs. 5 min)</td>
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<tr>
<td>Over 5 min (5 min vs. 10 min)</td>
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<tr>
<td>Over 10 min (baseline vs. 10 min)</td>
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<tr>
<td>Across all three images</td>
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</tbody>
</table>

95% CI 0.98, 0.99), and velocity (ICC = 0.98, 95% CI 0.96, 0.99). Image analysis methods using cursor options were less reliable than the AFFPIA full-field method.

Table 2 shows the intra-subject reproducibility of measures (using the AFFPIA full-field method) of multiple images per person, taken 5 min apart (baseline, 5 min, and 10 min later). An overall reproducibility of ICC 0.74 (95% CI 0.48, 0.90) was obtained.

DISCUSSION

The HRF has been used to measure blood flow in different diseases, such as glaucoma, diabetic retinopathy, and age-related macular degeneration among others, as it is well known that disturbances in retinal microvasculature are features of these diseases. Our results show that standard HRF software did not provide highly reliable measures of the SLDF parameters, but newer analysis software utilizing the full field of topographic images improves the reliability substantially. Using the image analysis mode with near-perfect reliability (AFFPIA full-field mode), we found an overall high reproducibility for capillary flow measurements.

Past research into HRF may not be directly comparable to our results due to the use of varied cursor sizes ranging from $2 \times 2$ to $50 \times 50$ pixels, the use of modified HRF devices with different types of laser heads (670 nm, default 780 nm, 790 nm), and different study designs or statistical methods. Iester and colleagues used AFFPIA software with full-field option, appropriate reliability experimental design, and calculated reliability as a coefficient of variation (CoV) ranging from 3.8–20.7% across different sections of the retina in a sample of healthy and glaucomatous eyes. Using a different statistical approach, our reliability of ICC 0.99 (95% CI 0.98–0.99) for capillary flow measured using AFFPIA full-field in healthy eyes is sufficiently high for repeated measures by the same observer, although this estimate is not directly comparable to the CoV values reported by other studies.

Many researchers have assessed reproducibility of results across subjects over time. In human subjects using HRF software, capillary flow reproducibility estimates have been reported (for the $10 \times 10$ pixel cursor) in the range of 0.82 (CoV) to 0.64 (ICC), where images were taken one day apart, and 91.4% (CoV) for images taken within 10 min. Using AFFPIA software with the full-field option, reproducibility estimates for capillary flow have been reported in the range of 0.4–1.9% for study designs where images were taken days apart, and 0.8–0.95 (ICC) for images taken within 30 min. Our results for reproducibility of ICC 0.74 over 5 and 10 min using AFFPIA full-field were close to those previous reports of ICC 0.8–0.95.

Reliability is affected by different image analysis factors, such as the choice of and size of measurement area and quality and clarity of the topographic image. The HRF takes 2 sec to capture a topographic image, working from the top left to the bottom right, the resultant image is subject to spatial heterogeneity in circulatory parameters due to heart beat-associated pulsations. As such, the placement of the cursor, or the choice and size of image area to be included in analysis, becomes important. Griesser and colleagues showed that the variation in HRF parameters decreased as the cursor size increased. The AFFPIA full-field option provides the highest reliability as it includes the largest possible image area for analysis, and automatically adjusts for the quality of the topographic image, “auto-cleaning” and masking any extra-wide vessels, optic nerve head, and over- or under-exposed pixels. So, for a given cursor size, we would expect the AFFPIA reliability to be better than for HRF software. All image analysis issues that affect reliability will affect reproducibility as well as imaging-related issues, such as choice of location within the eye itself, distance between camera and eye, room lighting, sharpness of topographic image, and changes within the subject, such as level of relaxation and ability to concentrate and fixate. The effect these imaging-related factors have on reproducibility will likely increase as the time between images increases.

The strengths of this study include the use of healthy subjects, with no eye pathology, who had not eaten...
or smoked for at least 3 hr and who rested prior to measurement by a single experienced grader. Possible limitations of the study include the small sample size (n = 13), the measurements not synchronized on the cardiac cycle, and the use of diffuse natural lighting rather than constant artificial lighting.14

In conclusion, we found that default HRF software, using a cursor-based method, does not provide reliable measures of capillary circulation parameters from HRF topographs. Newer image analysis software (AFFPIA) utilizing the full field of topographic images improves the reliability substantially and provides high intra-observer reliability for repeated measures on the same images, and high intra-subject reproducibility for measures on different images taken from the same subject over a short period of time. Therefore, we recommend using the AFFPIA full-field mode of image analysis in conjunction with the HRF camera for future research. Since SLDF is a relatively new technique, correlations between SLDF-measured capillary circulatory parameters and biologically plausible factors need to be assessed.

REFERENCES


