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Reference

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Reproducibility of the Retinal Vascular Response to Flicker Light in Asians

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ABSTRACT

Purpose: Dilation of retinal vessels in response to diffuse luminance flicker may reflect endothelial function. Although this has previously been shown to be reproducible in whites, there have been no similar data in Asians. We assess the reproducibility of repeated measurements of this response in Asians.

Material and Methods: Healthy Asians (n = 33) with normal vision and no history of glaucoma, age-related macular degeneration, cataract, or retinal arterial/venous occlusion participated in this study. Repeated measures from the same subjects were taken 30–60 min apart using the Dynamic Vessel Analyser (DVA, IMEDOS, Jena, Germany). Modification was made to the shape of the light source for Asian participants. Correlations of the first and second measures were assessed using Pearson correlation (R²), and agreement between the two measures was shown using Bland–Altman plots.

Results: After modification to the shape of the light source, almost perfect correlation was found between the 1st and 2nd measurements of baseline arteriolar (R² = 0.95) and venular diameters (R² = 0.98) of arteriolar maximum dilation (R² = 0.85). Substantially high correlation between the 1st and 2nd measurements of venular maximum dilation was found (R² = 0.80).

Conclusions: Measurements of the dilation response of retinal vessels to diffuse luminance flicker in an Asian sample using the DVA show high reproducibility for repeated measures over a short period of time. Such measurements may allow non-invasive quantification of endothelial function to study its association with systemic and ocular diseases.

KEYWORDS: Diffuse Luminance Flicker; Reproducibility; Retinal Vascular Imaging; Auto regulation, Vasodilation
microcirculation in various ocular and systemic diseases.

The Dynamic Vessel Analyser (DVA) has previously been used to measure the retinal vessel dilation in response to diffuse luminance flicker. Previous clinical studies using the DVA have shown the impaired dilation to this flicker, reflecting possibly endothelial dysfunction, is associated with systemic diseases, including hypertension and diabetes, and ocular diseases, such as diabetic retinopathy and glaucoma. However, although DVA has previously been shown to be reproducible in whites, there have been no similar data in groups/races other than whites, including Asians. In this study, we aim to assess the reproducibility of the retinal vessel dilation in response to flicker-light, as measured by the DVA in Asians and compare the corresponding reproducibility reported in whites.

MATERIALS AND METHODS

We included 33 Asian participants from the Centre for Eye Research Australia, University of Melbourne, Australia. The mean age is 34.1 (+13.3) years, and women accounted for 58% of the sample. 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 examination. Furthermore, the participants did not have a history of systemic or ocular diseases. Participants were not fasted. Measurements were obtained by one person (Thanh Nguyen).

DVA

Examination was conducted in a dark room. The participant focused on the tip of a fixation bar within the camera while the fundus was examined under green light. An arteriolar and venular segment of approximately 1.5 mm in length, between half and 2 disc diameters from the margin of the optic disc were selected (see Figure 1). The diameter of the vessel segments was calculated automatically. Baseline vessel diameter was measured for 50 sec, followed by a provocation with flicker light of the same wavelength for 20 sec, and then a non-flicker period for 80 sec. This measurement cycle was repeated twice, with a total duration of 350 sec. When the eye blinked, the vessel segments were not measured and measurement restarted once the vessel segments were automatically re-identified.

The measurements were assessed to ensure quality after each test. Good quality readings were those with minimal missing values and the diameter dilation patterns were similar over the three cycles (Figure 2A). If a cycle is of poor reading, then it was removed from the analysis. Retinal arteriolar and venular dilation in response to flickering light was calculated automatically by the DVA software, and defined as the average maximal increase in diameter of the three cycles during flicker-light stimulation. Manual calculation of the flicker-induced dilation was performed if there was only one cycle with good reading.

One eye was measured for each participant, and the same eye was re-measured 30–60 min later. Repetition mode was used during re-measurement to ensure that the same vessel segments were measured.

Modification of Shape of the Light Source

We have modified the shape of the light source of the DVA from a circular shape to a bandage-like shape to reduce the reflection of the light from the eyelids (Figure 3). This was necessary for Asians due to their relatively low vertical height of the palpebral fissure.

Statistical Analysis

Correlation of the 1st and 2nd measures were assessed by Pearson correlation ($R^2$) (Stata, Version 10.0, Stata Corp., College Station, TX, USA). $R^2$ values less than 0.40 represent poor to fair correlation, between 0.61 and 0.80 represent substantially high correlation, and 0.81 to 1.00 represent almost perfect correlation.
Bland–Altman plots were used to assess agreement between the 1st and 2nd measurements.\textsuperscript{18}

**RESULTS**

Figure 4 shows an example of the improvement of the readings after modification of the light source in a healthy 27-year-old Asian female. After modification, we can see proper flicker light induced dilation and a reduction of the missing data. The measurements shown were from different vessels (but of the same subject).

Measurements (from one session) from two Asians are unable to be evaluated due to poor quality and are excluded. Table 1 and Figure 5 show the correlation of the 1st and 2nd measurements of arteriolar/venular diameters at baseline and the maximum dilation. Almost perfect correlation is found between the 1st and 2nd measurements of baseline arteriolar (0.95) and venular (0.98) diameters and of arteriolar maximum dilation (0.85). Substantially high correlation between the 1st and 2nd measurements of venular maximum dilation was found (0.80).

Figure 6 shows the Bland–Altman plots of the differences between the 1st and 2nd measurements against the mean of the two measurements. The mean difference between the 1st and 2nd measurements of arteriolar maximum dilation is 0.0\%, and only 1 of 31 subjects (3.2\%) is outside the 95\% limits of agreement. The mean difference between the 1st and 2nd measurement of venular maximum dilation is 0.2\%, and 2 of 31 (6.5\%) are outside the 95\% limits of agreement.
DISCUSSION

Although DVA has previously been shown to be reproducible in whites, there have been no similar data in Asians. In this study, we show that the measurement of retinal vessels to flicker using the DVA has high reproducibility for repeated measures over a short period of time in Asians, similar to the reliability level previously obtained in whites with the use of the band-shape light source.

Recent studies showed that retinal vascular caliber sizes differed between whites and Asians, and thus speculated that the differences could have been resulted from different contrast of retinal background relatively to the retinal vessels between Asian and Caucasian eyes. Our findings did not provide evidence supporting such a speculation but documented that the amount of light exposed to the retina was likely influenced by the different vertical heights of the palpebral fissure between Asian and Caucasian eyes.

FIGURE 4 An example of the improvement of the readings after modification of the light source in a healthy 27-year-old Asian female. After modification, we can see proper flicker light induced dilation and a reduction of the missing data. The measurements shown were from different vessels (but of the same subject). Again, the y-axis is the diameter in Measurement Units which correspond to µm in the Gullstrand’s eye, and the x-axis is the time in seconds. In both situations described below, the venules are wider than the arterioles.
TABLE 1 Mean ± standard deviation of retinal vessel baseline diameters and maximum dilation of 1st and 2nd measurements, and Pearson correlation statistics ($R^2$)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>1st measurement</th>
<th>2nd measurement</th>
<th>Difference</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arteriole</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter*</td>
<td>117.6 ± 12.6</td>
<td>117.7 ± 11.3</td>
<td>-0.1 ± 3.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Maximum dilation (%)†</td>
<td>3.9 ± 1.8</td>
<td>3.9 ± 1.9</td>
<td>0.0 ± 1.0</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>Venule</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline diameter*</td>
<td>149.5 ± 18.4</td>
<td>149.9 ± 18.4</td>
<td>-0.5 ± 3.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Maximum dilation (%)†</td>
<td>5.4 ± 2.1</td>
<td>5.1 ± 2.2</td>
<td>0.2 ± 1.3</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*Measurement Units which correspond to µm in the Gullstrand’s eye.
†In % to individual baseline.

FIGURE 5 Plots of the 1st vs. 2nd measurements for baseline vessel diameters (Measurement Units which correspond to µm in the Gullstrand’s eye) and maximum dilation of retinal arteriole and venule. Solid lines represent linear regression, and dashed lines are lines of equality.
The reproducibility was improved substantially, with changing the shape of the light source, to be similar to that previously reported from whites. As the DVA has wide application potential, the modification of the light source and our study results have important implication for a wider application of this testing.

The endothelium plays a critical role in vascular physiology, and endothelial dysfunction has been suggested to play a pivotal role in the pathogenesis of cardiovascular diseases and ocular diseases, such as diabetic retinopathy. A major obstacle to clinical research of endothelial dysfunction is the difficulty in assessing it in vivo. Most measurements of endothelial function are time consuming and require highly specialized personnel and equipment. In contrast, the DVA doesn’t require much training, and the measurement can be performed in less than 15 min. The DVA quantifies non-invasively retinal vessel dilation in response to flickering light, which is believed to reflect endothelial function, given the documentation of the role of nitric oxide played in this flickering light induced retinal vasodilation.

The strength of this study is to extend the application of DVA beyond whites and confirm the same...
measurement validity of the DVA in Asians as that in whites. Possible limitations of this study include that the repeated measurements are taken over a short period of time, and our results are only applicable to healthy persons with healthy eyes and normal visual function. Furthermore, we are unable to say whether the variability between the readings is due to the technical limitations of the DVA machine or the biological/physiological variability in a human subject. In addition, we are uncertain whether this modification will also improve the results of DVA assessment in whites. This will be an area of future research.

In conclusion, we have demonstrated a high reproducibility of repeated measurements over a short period of time using the DVA to assess retinal vessel dilation in response to flickering light in Asians. The response of retinal vessels to diffuse luminance flicker has been suggested to reflect endothelial function. Thus, such measurements may allow non-invasive quantification of endothelial function to study its association with systemic and ocular diseases. Our findings assure this DVA technique is not only applicable to whites but Asians.

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REFERENCES