The Use of Fiji Image J as an Image Analysis Tool for Measuring Retinal Vessel Diameter in Rodent Model of Diabetic Retinopathy

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Abstract

The purpose of this study was to evaluate the use of the Fiji Image J application for digital fundus image analysis of retinal vessel diameter in a diabetic retinopathy rat model. Male Sprague-Dawley rats, weighing 200-250 grams, were divided into two groups: normal and diabetic. The diabetes was induced by an intraperitoneal (IP) injection of streptozotocin (STZ, 55 mg/kg body weight). Normal rats received IP citrate buffer. Fundus images were captured at week 0 (baseline), 6 and 12 post-induction to observe changes in retinal veins and arteries. Images obtained were then analyzed using Fiji Image J software (Version 1.53c, National Institute of Health, US). The retinal venous diameter was increased in both groups at week 6 and 12 compared to baseline (p<0.05). However, no significant differences were seen in the retinal venous diameter at week 12 compared to week 6 in both groups. When comparing between the groups, retinal venous diameter in the diabetic group was significantly greater compared to the normal group at week 6 and 12 by 1.37- and 1.35-folds (p<0.001), respectively. For the retinal arterial diameter in the diabetic group, an increase was observed at week 6 and 12 compared to baseline by 1.17- and 1.2-folds (p<0.05) respectively, however, similar changes were not observed in the normal group. There was also no significant difference between the retinal arterial diameter of the normal and diabetic group at week 6 and 12. In conclusion, retinal vessels diameter analysis of fundus images using Fiji Image J can be utilized to determine quantitative changes between normal and rats with STZ-induced diabetic retinopathy.

Keywords: Diabetic retinopathy, fundus imaging, retinal vessels diameter, streptozotocin, digital analysis

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Introduction

The higher prevalence of retinal diseases worldwide necessitates research with the use of experimental animal models to explore the pathophysiology and effects of novel treatment options. Fundus imaging is one of the important clinical parameters used in experimental research to assess the retinal morphology and to correlate with pathophysiological processes and functional outcomes. It allows real-time observation which can be correlated with retinal changes. Indirect ophthalmoscope using dioptre lens is one of the techniques used to observe the fundus. It is a low cost and non-invasive technique, and this is an advantage for non-specialized ophthalmology centres. Although human and bigger animals have widely been used, reports on the use of indirect dioptre lens for retinal imaging in small animals such as rats is limited. Notably, optical coherence tomography (OCT) is increasingly being used to capture fundus images. OCT has the advantage of creating cross-sectional images through its optical beam scanned through transverse direction, however, it is an expensive machine and is difficult to use in smaller animals.

The use of indirect ophthalmoscope together with the advanced smartphone camera is an attractive alternative, which is cheaper and yields high-quality fundus images that can be used for analysis. Fiji Image J application is an extension of Image J, which is an open-source software, developed by the National Institutes of Health (NIH). This software combines multiple and broader image-processing algorithms yet offering easy use for a beginner. In this short communication report, we describe the use of the Fiji Image J application to measure retinal vessels diameter from fundus images captured using a smartphone camera in a rat model of diabetic retinopathy.

Methodology

Animals

All experimental work was done in Laboratory Animal Care Unit (LACU) under animal ethical approval by the local institution (Ethical Approval No: UiTM CARE 3/2019/(286/2019)). Animals were handled following Associations for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research.

Male Sprague-Dawley rats (200-250 grams) were housed on a 12-hour light / dark cycle with access to food and water ad libitum. Rats were acclimatized for a week and underwent systemic and eye examination before commencing the study. Those found normal were included in the study.

Study Design

Rats were divided into two groups: normal control (N) and diabetic (D) rats. A total of 18 rats were used at the start of the study, of which eight rats were in N and 10 rats were in D group. Among D group of rats, two rats developed infection during experimental period and died. The remaining eight rats in D group survived until the end of the study.

Fundus images were captured at week 0, 6 and 12 post-diabetes induction to observe changes in retinal veins and arteries. Additionally, blood glucose and body weight were recorded once weekly throughout the experimental period.

Induction Of Diabetes

Rats were fasted overnight prior to intraperitoneal (IP) injection of streptozotocin (STZ) dissolved in an ice-cold sodium citrate buffer (10 mmol/L, pH 4.5) at a dose of 55 mg/kg body weight. Blood from the tail vein was collected 48 hours after induction to estimate blood glucose levels using the Accu Chek Performa glucometer (Roche Diagnostic, Basel, CH). Rats with blood glucose levels of more than 20 mmol/L were included in the diabetic group. Normal control animals received an IP injection of a sodium citrate buffer.

Fundus Imaging

Rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.; Nacalai Tesque, Kyoto, Japan) and tropicamide (MYDRIACYL® Alcon, Geneva, CH) was instilled onto the eye for pupillary dilatation 30 minutes prior to imaging. The optical disk was oriented to the centre of the view. Fundus images were captured with 3456×5184 pixels using a smartphone camera (Model: iPhone 7 Plus, Apple Technology Company, Cupertino, California, US) and the software Filmic Pro (Cinegenix LLC, Seattle, WA, USA; http://filmicpro.com/) with the aid of a dioptre lens for small animals (Model 78D; Volk Optical, Ohio, US). The dioptre lens was positioned in front of the rat’s eye with one cm gap between the cornea and the lens. The smartphone camera was kept about 8 cm from the lens. Fundus images were captured by two independent and blinded researchers at week 0, 6 and 12 post-diabetes induction. Around eight to ten fundus images were captured at each time point and those with clear arteries and venous images were selected for analysis.
Image Analysis

The fundus images from the smartphone camera were transferred into Fiji Image J software (Version 1.53c, National Institute of Health (NIH), Bethesda, MA, US) in 3456 x 4184 pixels, 1 µm per pixel, JPEG format for vascular diameter analysis. Poor quality images that showed less than three main retinal vessels, both veins and arteries, despite having the optic disc at the centre were excluded. The veins were recognized by their dark red colour with a broader calibre whereas, the arteries have a bright red colour with a smaller calibre [7].

The images were calibrated by fixing the diameter of the optic disc at 300 µm, which was based on the optic disc average diameter of rats reported by Cohan et al. [8]. The vessel diameter analysis process was adapted from Takai et al. [9]. An area within the distance of 0.5- and 1-disc diameter from the optical disc margin was demarcated. Three widest venous and three widest arteries that were situated in the demarcated zone between 0.5- and 1-disc diameter were measured in micrometre and their average values were then calculated.

Statistical Analysis

Statistical analysis of variance was performed using Independent Samples T-test and Paired T-test (SPSS v 24.0, SPSS Inc, Chicago, US). A p-value of less than 0.05 was considered significant. The results are presented as mean ± standard deviation of the estimates.

Results

![Figure 2: The representative fundus images of rats from the normal control (N) and diabetic group (D) at week 0, 6 and 12.](image)

The retinal venous diameter was greater in both groups at week 6 and 12 compared to the corresponding baseline. In the normal group, the diameter increased by 1.12- and 1.18- folds at week 6 and 12, respectively, compared to the corresponding baseline (p<0.01 and p<0.001 respectively), whereas, in the STZ-induced diabetic group, the diameter increased by 1.36- and 1.42- folds at week 6 and 12, respectively, compared to the corresponding baseline (both are p<0.001). The retinal venous diameter in both groups remained unchanged from week 6 to week 12. Intergroup comparison showed that retinal venous diameter in the STZ-induced diabetic group was significantly greater compared to the normal group at week 6 and 12 by 1.37- (p <0.001) and 1.35- folds (p<0.001), respectively (Figure 3).

![Figure 3: Retinal venous diameter in rats at week 0, 6 and 12 after experimental induction of diabetes. Rats were monitored for 12 weeks of the experimental period. N: Normal rats, D: Diabetic rats, n=8, **p<0.01; ***p<0.001.](image)
The retinal arteries showed an increase in the diameter in the STZ-induced diabetic group at week 6 (1.17-folds, p<0.05) and week 12 (1.2 folds, p<0.05) compared to the corresponding baseline. However, there were no changes in the arterial diameter of the normal group throughout the 12 weeks period. The intergroup comparison showed that there were no significant differences between arterial diameter in both groups throughout the period of 12 weeks (Figure 4).

![Figure 4: Retinal arterial diameter in rats at week 0, 6 and 12 after experimental induction of diabetes. Rats were monitored for 12 weeks of the experimental period. N: Normal rats, D: Diabetic rats, n=8, *p<0.05.](image)

**Discussion**

In this study, rat’s fundus images were captured using a smartphone camera with the aid of Filmic Pro application and the image produced were of high quality as described by Haddock et al. [5]. In this study, we used iPhone 7 Plus as our smartphone camera, however, Filmic Pro application can be utilized with any type of smartphone, which makes its use more flexible. The experimental conditions were standardized in terms of the distance between the phone camera, lens and the cornea of the rats and the camera/app setting were fixed as detailed in the methods section. In the rats with diabetes of 12 weeks duration, we did not observe any signs of pan-retinal haemorrhage, exudates or new vessels formation as is usually seen in diabetic retina of the human subject and this corroborated in earlier studies [10]. Previous studies, however, have shown increased tortuosity and diameter of the retina vessels in diabetic rats [11]. In this study, however, we did not observe visually evident tortuosity of the vessels, which may be due to the shorter duration of diabetes in this study compared to the previous study by Gupta et al. [11].

Fiji ImageJ software used in this study was able to analyse the retinal venous and arterial diameter and the results were comparable with the report from other studies, which used different analysis tools. We observed an increase of vessels diameter, particularly the venous diameter in diabetic rats compared to normal rats. An increase in venous diameter was evident in diabetic rats at 6 and 12 weeks post-STZ injection compared to the normal rats at the same time points. Previous studies have also reported similar observation when retinal vessel diameter was measured using transmission electron microscopy [12], flicker-induced dilation [13] and spectral-domain OCT [14] of the same model. The higher expression of retinal vascular endothelial growth factor (VEGF), in the diabetic retina, has been shown to contribute towards the increase in retinal vessel diameter, especially in the veins [15]. However, previous studies have reported contrasting findings concerning changes in the diameter of retinal vessel after experimental induction of diabetes in rats. Michoud et al. [16] and Gupta et al. [11] reported an increase in the diameter of retinal vessels of diabetic rats compared to normal control rats. However, Miyamoto et al. [17] and Wanek et al. [18] did not find any significant changes in the vessel diameter of diabetic rats compared to normal rats. These contrasting finding may be attributed to differences in the strains of animals used, duration of the study, the experimental procedure to induce diabetes and severity of hyperglycaemia [19]. For example, Bursell et al. [20] reported no increment in the retinal vessel diameter owing to the short duration of diabetes in their study.

**Conclusion**

In conclusion, we demonstrated the use Fiji ImageJ software to analyse the retinal venous and arterial diameter. This software provides easy-to-learn features based on existing works [6, 21, 22, 23] and is a cheaper option. Hence, it is useful for a novice researcher. Nonetheless, further studies are needed to validate this method against an established built-in OCT software or with trypsin digest protocol which is the gold standard method to analyse retinal vasculature.

**Conflict of Interest**

The authors would like to declare that there was no conflict of interest in this study.

**References**


