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Inter-visit variability of conjunctival microvascular hemodynamic measurements in healthy and diabetic retinopathy subjects

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ABSTRACT

Conjunctival microcirculation imaging provides a non-invasive means for detecting hemodynamic alterations due to systemic and ocular diseases. However, reliable longitudinal monitoring of hemodynamic changes due to disease progression requires establishment of measurement variability over time. The purpose of the current study was to determine inter-visit variability of conjunctival microvascular hemodynamic measurements in non-diabetic control (NC, N = 7) and diabetic retinopathy (DR, N = 10) subjects. Conjunctival microvascular imaging was performed during 2 visits, which were 17 ± 12 weeks apart. Images were analyzed to determine vessel diameter (D), axial blood velocity (V), blood flow (Q), wall shear rate (WSR) and wall shear stress (WSS). The inter-visit variability was determined based on mean inter-visit differences. In NC, inter-visit variability of D, V, Q, WSR and WSS were 0.2 ± 0.5 μm, −0.01 ± 0.16 mm/s, −8 ± 46 pl/s, $-3 \pm 46\ s^{-1}$ and -0.01 ± 0.10 dyne/cm^2, respectively. Inter-visit variability of D, V, Q, WSR and WSS were beyond the normal 95% confidence limits in 60%, 20%, 40%, 20% and 20% of DR subjects, respectively. The variability of hemodynamic measurements over time was established in non-diabetic subjects, suggestive of the potential of the method for detecting longitudinal changes due to progression of DR.

1. Introduction

The bulbar conjunctiva is a vascularized mucus membrane covering the outer layer of the eye. Conjunctiva has gained attention in the literature due to the ease of accessibility and visibility of blood flow within the microvascular network. Imaging modalities including orthogonal polarization spectral imaging (van Zijderveld et al., 2014), slit-lamp biomicroscopy (Jiang et al., 2014; Khansari et al., 2016b; Koutsiaris et al., 2007; Shahidi et al., 2010), and intravital microscopy (Cheung et al., 2002a; Cheung et al., 1999) have been developed for assessment of conjunctival microvascular hemodynamics. Furthermore, commercial devices such as retinal functional imager (Jiang et al., 2013) and Heidelberg retinal flowmeter (Duench et al., 2007) have been modified to measure conjunctival hemodynamics. Application of these imaging modalities has shown conjunctival microvasculopathy and hemodynamic alterations due to systemic diseases such as Alzheimer's disease (Smith et al., 2009), hypertension (To et al., 2013), hypotension (Gaynes et al., 2012), diabetes (Khansari et al., 2017; Cheung et al., 2009; Cheung et al., 2001; Owen et al., 2008; To et al., 2011), and sickle cell disease (Cheung et al., 2002a; Kord Valeshabad et al., 2015; Paton, 1962; Wanek et al., 2013). Furthermore, a recent study showed a significant decrease in conjunctival blood flow, vessel density and non-perfused areas in brain dead subjects as compared to normal controls (Tamosuitis et al., 2016). Moreover, abnormal conjunctival hemodynamics was reported during internal carotid artery surgery (Schaser et al., 2003).

The study of conjunctival microvasculature may help elucidate information relevant to the study of microcirculation in other human organs. Conjunctival microvascular complications due to diabetes have been reported (Cheung et al., 2009; Owen et al., 2008; To et al., 2011), similar to those reported in the retina (Ditzel, 1967; Tarr et al., 2013). Additionally, conjunctival blood flow has shown to be correlated with sublingual microcirculation in rats (Yin et al., 2016), and with cerebral blood flow in dogs (Ohtani, 1996).

Since systemic diseases can cause alterations in the conjunctival microvascular hemodynamics, studying inter-visit variability of the measurements is crucial to determine sensitivity for detection of changes due to diseases. Previous studies have reported inter-visit variability of blood flow in native arteriovenous fistula in chronic hemodialysis subjects and retinal vascular oxygen saturation in healthy

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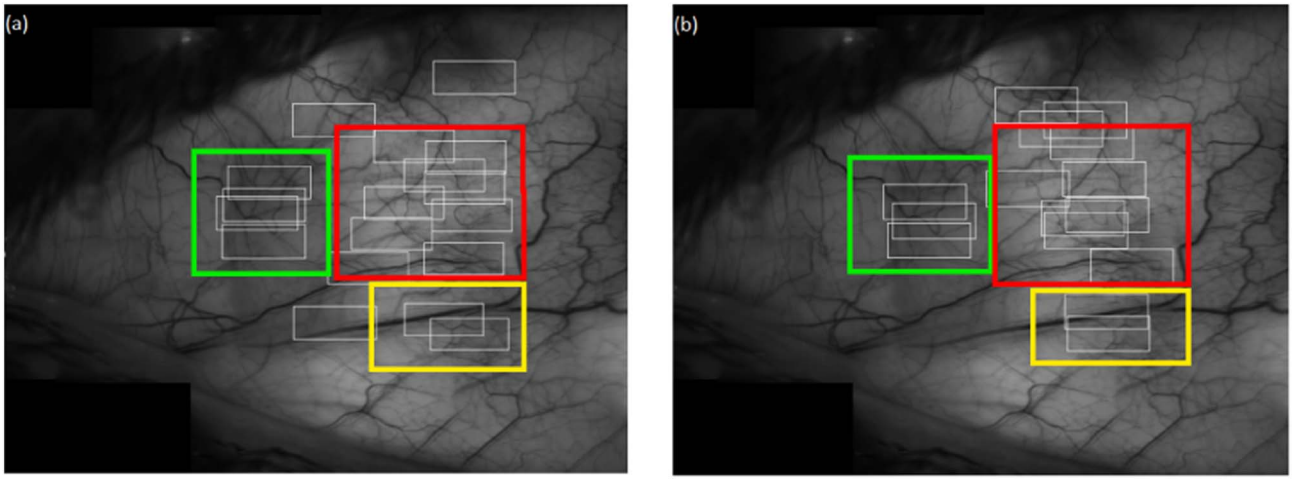

Fig. 1. Example of a conjunctival microvasculature mosaic image in an NC subject showing regions of image sequences (white boxes) for (a) the first and (b) the second visits. Overlapping regions between the 2 visits are shown by similarly colored boxes overlaid on the mosaic image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

subjects (O'Connell et al., 2014; Valek et al., 2008). In the conjunctiva, intra-visit variability of hemodynamics has been established within one or multiple sessions during a single day (Duench et al., 2007; Khansari et al., 2016b; Xu et al., 2015). Nevertheless, to the best of our knowledge, inter-visit variability of conjunctival microvascular hemodynamics was not reported previously. The purpose of the current study was to determine inter-visit variability of conjunctival microvascular hemodynamic measurements in non-diabetic subjects and report the incidence of longitudinal variations in diabetic retinopathy (DR) subjects.

2. Materials and methods

2.1. Subjects

This study was approved by an institutional review board of the University of Illinois at Chicago. The study was explained to subjects and informed consents were obtained according to the tents of Declaration of Helsinki. The study population included 17 subjects: 7 non-diabetic control (NC) (4 males and 3 females) and 10 DR (6 males and 4 females) subjects. Diagnosis was based on retinal examination performed by retinal specialists based on clinical examination. The exclusion criteria were stroke or myocardial infarction within 3 months of imaging, active angina, age-related macular degeneration, glaucoma, dry eye syndrome, retinal vascular occlusions, history of intraocular surgery, or cataract surgery within 4 months of imaging. Subjects' age were 36 ± 19 years (mean ± standard deviation (SD)) and 57 ± 12 years in NC and DR, respectively ($P = 0.01$). Before imaging, subjects were asked to sit for approximately 10 min to facilitate a cardiovascular and respiratory resting state. During imaging, subjects were seated in front of the slit lamp biomicroscope with chin and forehead support. An external fixation target was presented to the fellow eye to minimize eye movements. The same imaging protocol was performed at the follow-up visit. The follow-up durations were 11 ± 15 weeks and 22 ± 8 weeks in NC and DR subjects, respectively ($P = 0.06$). Data in one eye of each subject with repeated measurements in 3 or more vessel segments was included.

2.2. Image acquisition

Image acquisition was performed by our previously established non-invasive imaging system, EyeFlow (Khansari et al., 2016b). The system incorporated a slit lamp biomicroscope coupled with a digital camera. Imaging was performed on conjunctival regions temporal to the limbus. Several 1-second high magnification image sequences were recorded at 5.1× at a rate of 50 Hz (exposure of 20 ms). The high magnification images composed of 1360 × 550 pixels with a pixel resolution of 1.25 μm on the object plane. Contiguous low magnification images of conjunctival microvascular were acquired at 2× magnification. The low magnification images composed of 1024 × 1360 pixels with a pixel resolution of 3.12 μm on the object plane. The high and the low magnification images covered approximately a conjunctival region of 1.7 mm × 0.8 mm and 3.2 mm × 4.2 mm, respectively.

2.3. Image processing and analysis

High magnification image sequences were analyzed quantitatively using our previously developed automated method (Khansari et al., 2016b). In summary, on average 17 (range; 6–41) consecutive image frames were registered using an intensity based image registration technique to correct for eye movements. A mean image was generated by averaging the registered images. Different size conjunctival microvessels were then segmented using Frangi vesselness filter applied to the mean image. Vessel caliber (D) and axial blood velocity (V) measurements were obtained by full width at half maximum of intensity profiles and from the slope of prominent bands in the spatial-temporal images (STI), respectively. Average cross-sectional blood velocity (V_s) was computed from measurements of D and V. Blood flow ($Q = V_s \pi D^2 / 4$) and wall shear rate ($WSR = 8V_s / D$) were computed from V_s and D. Finally, wall shear stress ($WSS = \eta WSR$) was determined based on WSR and dynamic blood viscosity (η), where η was calculated from clinical hematocrit values as described previously (Koutsiaris et al., 2007; Shahidi et al., 2010).

2.4. Detection of repeated vessel segments

Conjunctival microvasculature regions imaged repeatedly were identified by generating a mosaic image and locating regions of imaging during each visit. The conjunctival mosaic image was generated per subject per visit as described previously (Khansari et al., 2016a). Briefly, contiguous low magnification images were processed semi-automatically using MosaicJ, a plug-in for ImageJ (ImageJ 1.48 V), to form a mosaic image. A human observer then used the best quality mosaic image from the 2 visits to locate conjunctival microvascular regions covered by each of the registered image sequences. Fig. 1 shows an example of the imaging regions (white boxes) in a NC subject at 2 visits. The mosaic image from the first visit was used to locate the repeated overlapping regions (color boxes).