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Feasibility of assessment of conjunctival microvascular hemodynamics in unilateral ischemic stroke

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ABSTRACT

Since the internal carotid artery supplies blood to both the eye and the brain, ocular microvascular hemodynamics can be altered due to ischemic stroke. The purpose of the current study was to establish the feasibility of conjunctival microcirculation imaging for detection of inter-ocular differences in microvascular hemodynamics in subjects with unilateral ischemic stroke. Conjunctival microcirculation imaging was performed in both eyes of 15 healthy control subjects and 12 subjects following unilateral ischemic stroke. Diameter and axial blood velocity were measured in multiple conjunctival venules of each eye. A two-way repeated measures analysis of variance was performed to determine the effects of stroke (control vs. stroke) and side of stroke (ipsilateral vs. contralateral) on conjunctival diameter and axial blood velocity. There was no significant main effect of stroke on conjunctival diameter ($P = 0.7$) or conjunctival axial blood velocity ($P = 0.9$). There was no significant main effect of side of stroke on conjunctival diameter ($P = 0.8$), but there was a significant main effect of side of stroke on conjunctival axial blood velocity ($P = 0.02$). There was a significant interaction effect between stroke and side of stroke ($P = 0.04$), indicating that conjunctival axial blood velocity was lower in ipsilateral eyes than in contralateral eyes of stroke subjects. Conjunctival axial blood velocity and internal carotid artery blood velocity were correlated in stroke subjects ($r = 0.75$, $P = 0.01$, $N = 10$). Conjunctival microcirculation imaging is a feasible method to detect inter-ocular differences in microvascular hemodynamics in subjects with unilateral ischemic stroke.

Introduction

Stroke is the third leading cause of death and the leading cause of long-term disability in the United States. A common cause of ischemic stroke is insufficient blood supply from the internal carotid artery (ICA) to one side of the brain. Currently, vascular imaging techniques are available for evaluation of ICA stenosis and blood velocity in subjects with ischemic stroke (Bleeker et al., 2012; Gough, 2011; Marquering et al., 2012; Rodriguez et al., 2011; Zachrisson et al., 2012). Since oxygenated blood is supplied to the eye and brain by the ICA, assessment of the ocular microcirculation may provide useful information about the cerebral blood supply. Previous studies have established relationships between conjunctival and cerebral blood flow in dogs (Ohtani, 1996) and in humans during aortic arch surgery (Schaser et al., 2003). Furthermore, alterations in conjunctival blood flow due to ICA occlusion have also been reported (Pavlou and Wolff, 1959). Recently, several studies have demonstrated retinal microvascular changes to be associated with, or predictive of stroke (De Silva et al., 2011; Doubal et al., 2009; Ikram et al., 2006; McGeechan et al., 2009; Ong et al., 2013; Wang et al., 2007; Wieberdink et al., 2010; Wong et al., 2001). Since the retina and conjunctiva have a common source of blood from the ophthalmic artery and analogous alterations in the retinal and conjunctival microvasculature have been reported in hypertension and diabetes (To et al., 2013; To et al., 2011), it is likely that the conjunctival microcirculation is also affected by stroke. The purpose of the current research study was to evaluate the feasibility of our conjunctival microcirculation imaging method (Gaynes et al., 2012; Kord Valeshabad et al., 2014; Shahidi et al., 2010; Wanek et al., 2013) for detection of inter-ocular hemodynamics differences due to unilateral ischemic stroke.

Materials and methods

Subjects

The research study was approved by an Institutional Review Board at the University of Illinois at Chicago. Prior to enrollment, the research study was explained to the subjects and informed consents were

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obtained according to the tenets of the Declaration of Helsinki. Fifteen healthy control subjects (male 7, female 8) without a history of cerebrovascular, hypertension or ocular diseases and 12 subjects (male 7, female 5) with a clinical diagnosis of unilateral ischemic stroke participated in this study. The median time interval between the occurrence of stroke and conjunctival microcirculation imaging was 30 days (range: 2–233 days). Exclusion criteria were inability to consent, hemorrhagic stroke, hypertension (blood pressure ≥140/90 mmHg), intracranial aneurysms that required surgery, sickle cell disease, history of eye diseases, ocular surface conditions, and eye drop treatment, or use of local sympathomimetic or para-sympatholytic medications prior to conjunctival imaging. The diagnosis of stroke was confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI). Transthoracic and transesophageal echocardiogram reports, telemetry evaluations and ICA narrowing, as determined by Doppler ultrasound, MRA, CTA or digital subtraction angiography, were recorded from clinical charts. Bilateral ICA blood velocity measurements using Doppler ultrasound were available in 5 of 12 stroke subjects. The median time interval between the Doppler ultrasound and conjunctival microcirculation imaging in these 5 subjects was 44 days (range: 4–232 days). The mechanism of stroke was determined based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (Adams et al., 1993) by one of the manuscript authors (FDT) who was masked to the conjunctival imaging data. Systolic and diastolic blood pressure (SBP, DBP) and heart rate (HR) were measured at the time of conjunctival microcirculation imaging and 3 repeated measurements were averaged. Demographic and clinical data were compiled in all subjects.

Image acquisition

Conjunctival microcirculation imaging was performed with the use of our optical imaging system (EyeFlow™) as previously described (Shahidi et al., 2010; Wanek et al., 2013). In brief, a slit lamp biomicroscope was utilized to visualize the conjunctival microcirculation, while subjects were seated with their head secured by the chin and forehead support, and presented with an external fixation target to minimize eye movement during imaging. A narrow band filter with a transmission wavelength of 540 ± 5 nm, which corresponds to a hemoglobin absorption peak, was placed in the path of the slit lamp illumination light to improve contrast of the microvasculature and visualization of red blood cells. Several 2-second image sequences, displaying red blood cell movement within the conjunctival microcirculation, were captured by a digital charged coupled device camera attached to the slit lamp biomicroscope. The frame rate of the camera was 30 Hz, thereby 60 images were acquired in each image sequence. In order to minimize the potential effects of variable light exposure and heating of the conjunctival surface on hemodynamics measurements, image acquisition was standardized in all subjects. The light levels were kept constant by using identical settings on the slit lamp illumination, placing a heat absorbing filter (ThorLabs Inc., Newton, New Jersey, USA) in the light illumination path, and restricting the light exposure time to approximately 15 min. In all subjects, images were obtained from both eyes at multiple locations that were temporal to the limbus. In stroke subjects, fellow eyes were designated as ipsilateral (IPSI) or contralateral (CONTRA) according to the side of stroke. The individuals who performed conjunctival imaging and image analysis were masked to the side of the stroke in the subjects.

Image analysis

The image analysis method for deriving measurements of conjunctival diameter and axial blood velocity has been previously described and validated (Kord Valeshabad et al., 2014; Shahidi et al., 2010). In each conjunctival microcirculation image sequence, 10 or more consecutive image frames were manually selected for analysis based on image quality and the absences of blinks and large eye movements. Image registration was then performed on these selected frames to correct for eye motion using a semi-automated, area-based search technique that employed control points, as previously described (Shahidi et al., 2010). From the registered conjunctival microcirculation images, a venule of interest was identified and the centerline was automatically extracted by selecting the end points of the vessel segment and using distance transform to obtain a line between the points. Conjunctival diameter was derived as the full width at half maximum of intensity profiles generated perpendicular to the vessel centerline, averaged along the vessel length. Along the venule centerline, motion of aggregated red bloods cells (or plasma gaps) was tracked in consecutive frames of the registered image sequence to generate a spatial–temporal image that displayed the intensity variation along the length of the vessel as a function of time. Conjunctival axial blood velocity was derived by determining the slope of the prominent bands in the spatial–temporal using 1D cross-correlation between the intensity data in the columns of the space time image. The spatial–temporal images were indirectly calibrated based on the known image acquisition frame rate and the spatial resolution of the imaging system (Shahidi et al., 2010). These values defined the x- and y-axis of the spatial–temporal images, such that the determination of the slope in the image yielded an accurate measure of conjunctival axial blood velocity.

Fig. 1 displays an example of a conjunctival microcirculation image and a spatial–temporal image (insert) obtained in one eye of a subject with unilateral ischemic stroke. Conjunctival axial blood velocity was measured from the spatial–temporal image as the slope of the overlaid red line which was equal to the slope of all prominent bands. In this example, conjunctival diameter and axial blood velocity in the selected venule were 17.6 μm and 0.33 mm/s, respectively. Diameter and axial blood velocity measurements were obtained in 4 or more venules in each eye and an averaged value was then calculated. The median number of venules that were analyzed in each eye was 11 venules (range: 4–20). Venules were distinguished from arterioles by visualizing the motion of red blood cells within the vessel and determining whether blood drained into another vessel (venule) or diverged into vessel branches (arteriole). Conjunctival venules were selected for hemodynamic

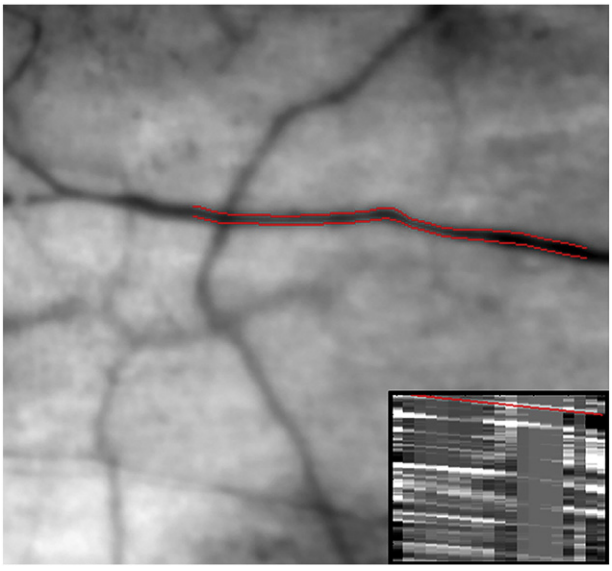

Fig. 1. Example of a conjunctival microcirculation image obtained in one eye of a subject with unilateral ischemic stroke. The outlined edges of the selected conjunctival venule (red lines) were automatically identified from diameter measurements. A spatial–temporal image (insert) was derived from this vessel based on the movement of aggregated red blood cells or plasma gaps along the vessel length. Conjunctival axial blood velocity was measured as the slope of the overlaid red line which was equal to the slope of the prominent bands within the image.