Correlation of abnormal intracranial vessel velocity, measured by transcranial Doppler ultrasonography, with abnormal conjunctival vessel velocity, measured by computer-assisted intravital microscopy, in sickle cell disease


The Stroke Prevention Trial has confirmed that utilization of transcranial Doppler ultrasonography (TCD), which examines blood flow in large intracranial vessels, can identify children with sickle cell disease (SCD) who are at high risk of developing a premature stroke. It is not known to what extent the vasculopathy in SCD involves small vessels and whether the abnormalities, if present, correlate with large-vessel vasculopathy. Eighteen children with SCD were examined with TCD to determine middle cerebral artery (MCA) velocity and computer-assisted intravital microscopy (CAIM) to determine bulbar conjunctival vessel velocity during the same visit for vasculopathy correlation. High MCA velocity (>200 cm/sec) was found by TCD in 4 patients who also showed abnormal conjunctival velocity (<0.2 mm/sec or intermittent trickle flow) by CAIM. Three patients had conditional (≥170 cm/sec and <200 cm/sec) MCA velocity: 2 showed abnormal (trickle) and 1 showed normal conjunctival velocity (1.9 mm/sec). One patient with unmeasurable MCA velocity had abnormal (trickle) conjunctival velocity. Of the remaining 10 patients who had normal MCA velocity, 2 showed abnormal (0.05 mm/sec and 0.1 mm/sec) and 8 showed normal conjunctival velocities (1.1-2.4 mm/sec). The MCA velocities correlated significantly with bulbar conjunctival flow velocities (P ≤ .008, Fisher exact test). A correlation exists between MCA (large-vessel) and conjunctival (small-vessel) flow velocities. CAIM is a noninvasive quantitative technique that might contribute to the identification of SCD patients at high risk of stroke. Small-vessel vasculopathy might be an important pathological indicator and should be further explored in a large-scale study. (Blood. 2001;97:3401-3404)

Introduction

Cerebrovascular disease, the most serious complication in homozygous (HbSS) sickle cell disease (SCD) patients, most often results from occlusion or reduction of cerebral blood flow of major intracranial arteries.1,2 Cerebral infarction with acute neurologic deficits affects 5% to 17% of SCD patients by 15 years of age.1,4 The Cooperative Study of SCD recently reported the chances of having a first cerebrovascular accident by 20, 30, and 45 years of age were 11%, 15%, and 24%, respectively, for HbSS patients.7 Using magnetic resonance imaging, it has been demonstrated that up to 34% of HbSS patients who did not have a clinically apparent neurologic event had silent cerebral infarctions often associated with neuropathological impairments.8,9 Cerebral infarctions and strokes in SCD often result from occlusion of major intracranial vessels and reduction of cerebral blood flow.10

Transcranial Doppler ultrasonography (TCD) has been used to measure blood flow velocity in the intracranial arteries of the circle of Willis, including the internal carotid artery (ICA) and the middle cerebral artery (MCA).10,11 Focal elevation of velocity, via TCD measurement, usually indicates arterial stenosis because flow velocity is directly related to cerebral blood flow and inversely related to arterial diameter.10 The Stroke Prevention Trial in Sickle Cell Anemia (STOP) has confirmed that utilization of TCD to identify high MCA velocity is predictive of high risk and vulnerability for stroke in children with SCD.2,3,8,10 Small vessels can readily be noninvasively assessed in the bulbar conjunctiva, but it is not known whether large-vessel (MCA) vasculopathy correlates with small-vessel (conjunctival) abnormalities.

Computer-assisted intravital microscopy (CAIM) has recently been developed as an objective quantitative technology to study vasculopathy in small vessels, using the readily accessible microcirculation of the bulbar conjunctiva as a noninvasive research site.12-14 It has been used successfully to assess and quantify the improvements in diabetic microangiopathy in diabetic patients after successful simultaneous pancreas-kidney transplantation.12 This report describes a correlative study on the vasculopathy of large and small vessels in the same SCD patients, using TCD to measure MCA (large-vessel) velocities and CAIM to measure conjunctival vessel (small-vessel) velocities.
Table 1. Correlation of hematologic characteristics with middle cerebral artery velocities (transcranial Doppler ultrasonography)

<table>
<thead>
<tr>
<th>MCA velocity (TCD)</th>
<th>Hemoglobin, g/dL</th>
<th>Reticulocytes, %</th>
<th>F-hemoglobin, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>High (n = 4)</td>
<td>7.8 ± 0.7</td>
<td>11.8 ± 2.6</td>
<td>7.5 ± 3.4</td>
</tr>
<tr>
<td>Conditional (n = 3)</td>
<td>7.7 ± 0.8</td>
<td>10.3 ± 3.1</td>
<td>7.9 ± 0.6</td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>8.7 ± 1.0</td>
<td>7.5 ± 3.2</td>
<td>8.6 ± 3.4</td>
</tr>
<tr>
<td>Unmeasurable (n = 1)</td>
<td>7.6</td>
<td>6.9</td>
<td>7.3</td>
</tr>
</tbody>
</table>

P = NS

NS, nonsignificant.

Patients and methods

SCD patients

SCD patients who were 5 to 18 years of age and had been given a diagnosis of HbSS were recruited for the study. Patients were excluded from the study if they had a history of stroke or were transfused within the previous 2 months. Informed consent was obtained from the patients’ parents or guardians.

Research design

Eighteen children with SCD were screened for exclusion criteria for the study at Children’s Hospital at Oakland, CA (CHO). Two non-SCD subjects, unknown to the investigators, were also included in the study to assess the reliability of the investigators to blindly identify the normal conjunctival microcirculation of non-SCD subjects. TCD recordings measuring MCA velocities were made and sent in diskette format to the STOP TCD Reading Center at the Medical College of Georgia at Augusta, GA, for blinded velocity determination. The results were categorized into 1 of 4 exclusive categories: normal (<170 cm/sec time-averaged mean velocity), conditional (≥170 cm/sec and <200 cm/sec), high or abnormal (≥200 cm/sec), and unmeasurable. The conjunctival microcirculations of the same patients were videotaped by CAIM during the same visit for subsequent conjunctival vessel velocity determination, with the patients’ names and medical records blinded to the investigators conducting the videotaping procedure. The videotape sequences of all patients were coded and sent to UC Davis to be objectively analyzed off-site for flow velocity measurement. The TCD measurements for MCA velocities from the STOP TCD Reading Center and CAIM measurements for conjunctival vessel velocities from UC Davis were sent directly to CHO, where the codes were matched for data correlation.

Transcranial Doppler ultrasonography

TCD measurements of MCA velocity were made following a protocol similar to that used in adults, but modified for children with SCD. A 2-MHz pulsed Nicolet Doppler Ultrasound, model EME TC2000 (Madison, WI), was used. The highest time-averaged mean blood flow velocities in 2-mm increments in the MCA (at 3 points) were recorded for each SCD patient. Experts assigned by the STOP TCD Reading Center to read the coded TCD diskettes were unaware of the patient’s medical record, identity, location, or prior TCD results. The diskettes were read, and the MCA velocities were identified as normal, conditional, high or abnormal, or unmeasurable. The assignment of at least 200 cm/sec as high MCA velocity was taken from Adams et al and was associated with a 40% risk of stroke within 3 years.

CAIM

Under CAIM, the conjunctival vessels appear as crisp black lines or tubes on a white background. In any video frame showing the conjunctival microvasculature, active blood flow was always visible in at least some, if not most, of the conjunctival vessels. Normal blood flow could range widely, and the flow velocities in different vessels could vary considerably even in the same video frame. However, the conjunctival blood flow velocity of an SCD patient would be considered normal if the averaged flow velocity of the patient was computed via image analysis to be more than 0.3 mm/sec (historical median steady-state velocity for HbSS patients at the UC Davis Sickle Cell Clinic was 1.6 mm/sec; n = 30 [A.T.W.C., unpublished data, 1998-2000]). The steady-state conjunctival velocity of any SCD patient would be considered abnormal if the flow velocity fell below 0.3 mm/sec. At times, one could see the conjunctival blood flow reduced to a trickle in SCD and diabetic patients (with recognizable sluggish red blood cell translocation—box car phenomenon); in such cases, the velocities could not be reliably computed and were reported as abnormal intermittent trickle flow.

The CAIM procedure has been adapted to noninvasively study the conjunctival microcirculation in human subjects. To record the conjunctival blood flow for objective measurement, a charge-coupled device video camera (COHU model CCD-6415-3000; San Diego, CA) was used for image acquisition via videotaping using CAIM. All videotapes were viewed in their entirety. Videotape sequences to be analyzed were chosen and coded for subsequent analysis. The imaging system was PC-based, equipped with an imaging board/frame-grabber (Data Translation model DT2851; La Habra, CA), and was put on-line with a video system via a FOR-A timer-integrator (FOR-A model VTG-33; Scientific Instruments, Sunnyvale, CA). Video images were frame-captured, digitized, and quantified for conjunctival microvascular characteristics including morphometry and flow velocity via in-house–developed imaging software: VASCAN using a nearest neighboring averaging and local thresholding with subsequent thinning algorithm and VASVEL using a single-step acquisition multiple-frame tracing algorithm. An area of 8.53 mm² on the bulbar conjunctival surface of each captured frame was used for data analysis. At least 5 video sequences from each patient (using 1 frame for each morphometric measurement and 8 successive frames for each velocity measurement per sequence) were used. Morphometric (diameter, vessel density, and distribution) and dynamic (velocity) measurements from all 5 sequences of each patient were averaged.

Hematology

Venous blood was obtained from each patient for the determination of hemoglobin level, reticulocyte count, and fetal hemoglobin (F-hemoglobin) measurement. All hematologic measurements were made at the hematology laboratory at CHO by standard methods.

Statistics

Statistical analysis was performed using Systat Version 8 (SPSS, Chicago, IL), and the averaged results were presented as the mean and SD whenever appropriate. Comparison of MCA velocities with conjunctival blood flow abnormalities were made using the Fisher exact test. Correlation of hematologic parameters with TCD and CAIM velocities was conducted using analysis of variance (ANOVA). A significance level of .05 was adopted for all analysis. Positive and negative predictive values were calculated using standard equations, considering high and conditional MCA velocities to be abnormal and excluding the unmeasurable MCA velocity.

Results

A total of 18 SCD patients and 2 non-SCD subjects were studied. The video sequences of their conjunctival microcirculations were
coded and analyzed blindly. Using morphometric characteristics (vessel diameter, vessel density and distribution, vessel tortuosity, arteriole:venule ratio, sludging, box car phenomenon, and damaged vessel and hemosiderin deposit) as criteria, the 2 non-SCD subjects and the 18 SCD patients were identified correctly.

The 18 SCD patients had the following (mean ± SD) hematologic values: hemoglobin (8.2 ± 0.9 g/dL), reticulocyte count (9.1% ± 3.3%), and F-hemoglobin (8.5% ± 3.2%). These values were consistent with SCD values in the literature and did not differ significantly within MCA velocity or conjunctival velocity categories (Tables 1 and 2). None of the patients with high or conditional MCA velocities have experienced a stroke in the 3 years following the study. Of the patients confirmed with high MCA velocities, 2 have subsequently been started on hydroxyurea therapy and 1 was put on hydroxyurea therapy after initial chronic transfusion treatment.

The values of MCA velocities measured by TCD and conjunctival velocities measured by CAIM for the 18 SCD patients were tabulated and correlated (Table 3). High MCA velocity (201, 204, 201, and 207 cm/sec) was found in 4 SCD patients. The same 4 patients also showed significantly abnormal conjunctival blood flow velocities (0.1, 0.1, and 0.2 mm/sec and intermittent trickle flow) compared with normal steady-state SCD velocities in this study (1.1-2.4 mm/sec) and historical steady-state SCD velocities in this laboratory (A.T.W.C., unpublished data, 1998-2000) (P < .001, ANOVA). Three SCD patients had conditional (193, 171, and 191 cm/sec) MCA velocities; 2 of the 3 showed abnormal (trickle) conjunctival velocities, while the remaining 1 showed normal conjunctival velocity (1.9 mm/sec). One SCD patient who had unmeasurable MCA velocity showed abnormal conjunctival velocity (trickle). Of the remaining 10 SCD patients who had normal MCA velocities (< 170 cm/sec), 2 showed abnormal (0.05 mm/sec and 0.1 mm/sec) and 8 showed normal steady-state SCD conjunctival velocities (1.1-2.4 mm/sec). The MCA velocities correlated significantly with the bulbar conjunctival velocities in this study (P ≤ .008, Fisher exact test). Conjunctival velocity demonstrated a positive predictive value of 75% and a negative predictive value of 80% for abnormal MCA velocity.

### Discussion

Identification of SCD children at high risk for stroke is important, because this is the major criterion for initiating chronic red blood cell transfusion and is also a criterion for bone marrow transplantation. Because these therapies involve significant risks, a high predictability of future stroke is necessary to justify their selection. In the STOP study, TCD was used to identify SCD children at high risk for strokes. Using survival analysis, Adams et al clearly showed that SCD patients having MCA or ICA velocities more than 200 cm/sec have a 40% chance of having a stroke during a 40-month period, while patients with velocities between 170 and 200 cm/sec had only a less than 7% chance of stroke during the same period. Using these data as basis, Adams et al selected an MCA or ICA velocity of at least 200 cm/sec for inclusion in a recently completed study that demonstrated the efficacy of chronic transfusion in preventing first stroke in children with SCD.

Despite demonstrating a high correlation of high intracranial vessel (MCA and ICA) velocity with the risk of stroke, Adams et al also identified SCD children in the normal velocity range (< 170 cm/sec) who experienced strokes. In a study of 315 SCD children, strokes occurred in 17 patients. While most (n = 12) of the SCD patients experiencing a stroke had intracranial vessel velocity values of more than 200 cm/sec, 5 children with velocities less than 170 cm/sec also had ischemic cerebral infarctions. Two of these 5 children had MCA velocity measurements in the very low to unmeasurable category. One of these 5 children had normal MCA velocity but was shown to have 75% to 99% stenosis of the right ICA and occlusion of the left ICA. A subgroup of patients with normal, low, or unmeasurable velocities are clearly at risk for stroke. Adams et al suggested that low TCD measurements could result when the angle of insonation is greater than 0 or from a poor temporal acoustic window. In addition, they noted that the intracranial velocities measured by TCD might decrease when the stenosis reached a critical level or might, in fact, be absent (unmeasurable) when total or close to total occlusion has occurred. In this study, we have identified 3 patients (1 with unmeasurable MCA flow velocity and 2 with normal MCA velocity) who have abnormal conjunctival velocity and may represent subgroups at high risk for stroke without high MCA velocities.

There is a significant correlation (P ≤ .008, Fisher exact test) between large- and small-vessel vasculopathy; we have shown that the conjunctival blood flow slowed to a trickle (≤ 0.2 mm/sec or intermittent trickle flow) in all SCD patients (n = 4) with high MCA velocity (≥ 200 cm/sec), 2 patients with conditional MCA velocity (> 170 cm/sec and < 200 cm/sec), and 1 patient with unmeasurable MCA velocity. It is possible that detailed analysis of the vessel morphometry of the bulbar conjunctiva or perfusion characteristics of the conjunctival surface, as has been performed by the same CAIM technology in other microvascular studies, may improve our ability to discriminate subgroups within these high-risk/stroke-vulnerable patient categories and direct preventive therapy at the individuals at greater risk. This correlative study, though small in the number of patients studied, supports a wider application of this objective and quantitative CAIM technology for correlation with TCD, magnetic resonance imaging, and clinical outcome measures.

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References

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