Migraine and Biomarkers of Endothelial Activation in Young Women

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Background and Purpose—There is mounting evidence of endothelial activation and dysfunction in migraine. Our objectives were to determine in a population of premenopausal women whether endothelial activation markers are associated with migraine.

Methods—Women (18 to 50 years) with and without migraine and free from cardiovascular disease were evaluated with tests of coagulation (von Willebrand factor activity, prothrombin fragment), fibrinolysis (tissue-type plasminogen activator antigen), inflammation (high-sensitivity C-reactive protein), and oxidative stress (homocysteine, total nitrate/nitrite concentrations, thiobarbituric acid–reactive substances).

Results—Sixty-one participants had migraine with aura (MA), 64 had migraine without aura (MO), and 50 were controls. Compared with controls, women with migraine had higher adjusted odds ratios for elevated von Willebrand factor activity of 6.51 (95% CI, 1.94 to 21.83) in those with MA and of 4.59 (95% CI, 1.37 to 15.38) in those with MO, elevated high-sensitivity C-reactive protein of 7.99 (95% CI, 2.32 to 27.61) in those with MA and of 2.63 (95% CI, 0.73 to 9.45) in those with MO, and for lower nitrate/nitrite levels of 6.60 (95% CI, 2.06 to 21.16) in those with MA and of 3.03 (95% CI, 0.90 to 10.15) in those with MO. Within the migraine group, von Willebrand factor activity was correlated with tissue-type plasminogen activator antigen (P=0.035) and nitrate/nitrite (P=0.024). There was a trend with high-sensitivity C-reactive protein (P=0.09).

Conclusions—In premenopausal women with migraine, particularly in those with MA, there is evidence of increased endothelial activation, a component of endothelial dysfunction. (Stroke. 2009;40:2977-2982.)

Key Words: migraine ■ stroke ■ endothelial activation ■ endothelial dysfunction ■ von Willebrand factor ■ C-reactive protein

Migraine is a risk factor for stroke, especially in women of reproductive age and in those with aura (MA).1 The mechanisms to account for the migraine-stroke association have been elusive, as traditional cardiovascular risk factors are often absent.2 There is, however, mounting evidence that migraine is linked to endothelial dysfunction, a condition referred to as “the ultimate risk of the risk factors,”3 and that is predictive of an increased rate of cerebrovascular and cardiovascular events. Endothelial dysfunction, a process mediated by oxidative stress, is demonstrated by endothelial activation and impaired vascular reactivity. Endothelial activation is characterized by a procoagulatory and proinflammatory milieu,4 as has been the subject of reports in migraine,5 but few studies have concentrated on premenopausal women, the population most affected by migraine6 and by migraine-associated stroke.7 The recent finding of decreased circulating endothelial progenitor cells in migraineurs suggests an increased need for repair due to endothelial perturbation.8 Endothelial progenitor cell determination can only be performed on fresh specimens. Plasma and urine biomarkers, however, lend themselves to being frozen and stored for future analysis.

Our objective in this study was to determine whether plasma and urinary biomarkers related to endothelial activation are associated with migraine and with MA within the population most prone to stroke, ie, premenopausal women. Markers were assessed in the areas of coagulation (von Willebrand factor [vWF] activity, prothrombin fragment [F1+2]), fibrinolysis (tissue-type plasminogen activator [t-PA] antigen), inflammation (high-sensitivity C-reactive protein [hs-CRP]), and oxidative stress (homocysteine, total nitrate/nitrite concentration, and thiobarbituric acid–reactive substances [TBARS]).

Subjects and Methods

Study Population

This study was conducted between February 2006 and October 2008 after approval by the institutional review board. Participants were recruited from advertisements in the ambulatory Headache Center, university campus website, institution-wide e-mail, radio, and the
local newspaper. Within the Headache Center, participation was offered to consecutive eligible patients after evaluation by the principal investigator (PI). Each prospective candidate was interviewed and examined by the PI to determine eligibility. The enrollment plan was to include equal numbers of migraineurs with (MA) and without (MO) aura, in addition to nonmigraine controls. Inclusion criteria for migraineurs were as follows: (1) women with MA or MO, as defined by criteria set forth in the International Classification for Headache Disorders-II (codes 1.1, 1.2, 1.5); (2) age 18 to 50 years; and (3) headache-free for at least 7 days at the time of enrollment. Potential control subjects who matched the ages of the cases (based on 5-year group intervals) were screened for enrollment according to a standardized questionnaire to determine eligibility. Exclusion criteria were as follows: (1) not physically well enough to give blood; (2) presence of diabetes mellitus, vasculitis, prior stroke/transient ischemic attack, pregnancy (self-reported), myocardial infarction, or systemic lupus erythematosus; (3) use of anticoagulants; (4) use of nonsteroidal anti-inflammatory drugs or other antiplatelet agents in the week before testing; and (5) not literate in English.

Clinical Information
Participants completed a questionnaire regarding age, education, household income, height, weight, age of headache onset, headache-related disability, physician-diagnosed medical conditions (including hypertension, smoking, hyperlipidemia, history of deep venous thrombosis, pulmonary embolism). The PI supplied the following information when applicable: International Classification for Headache Disorders-II headache diagnoses, average monthly days with headache-free for 1 week. Urine and blood were collected between 8 and 9 AM. Blood was drawn without a tourniquet. Analysis was performed blinded to participants’ health or laboratory information. Blood was drawn into collection tubes with 3.2% buffered sodium citrate for vWF activity, the inflammation marker hs-CRP and some of the coagulation markers, t-PA antigen, and F1+2 assays. Coagulation assays were performed by Esoterix, Inc, Laboratory Services, Aurora, Colo. The vWF activity assay uses plasma vWF to agglutinate platelets in the presence of ristocetin. By comparing the rate of agglutination against a normal reference curve, the vWF activity as a percentage was quantified. t-PA antigen and F1+2 assays were performed by ELISA. The hs-CRP assay was done with the nephelometry technique.

Laboratory Methods
All testing was done after the subjects had not taken nonsteroidal anti-inflammatory drugs or other antiplatelet agents for at least 1 week and after an overnight fast. Those with migraine needed to be headache-free for 1 week. Urine and blood were collected between 8 and 9 AM. Blood was drawn without a tourniquet. Analysis was performed blinded to participants’ health or laboratory information. Blood was drawn into collection tubes with 3.2% buffered sodium citrate for vWF activity, the inflammation marker hs-CRP and some of the coagulation markers, t-PA antigen, and F1+2 assays. Coagulation assays were performed by Esoterix, Inc, Laboratory Services, Aurora, Colo. The vWF activity assay uses plasma vWF to agglutinate platelets in the presence of ristocetin. By comparing the rate of agglutination against a normal reference curve, the vWF activity as a percentage was quantified. t-PA antigen and F1+2 assays were performed by ELISA. The hs-CRP assay was done with the nephelometry technique.

Blood was collected in serum-separator tubes for measuring levels of serum total cholesterol and TBARS. Cholesterol levels were measured by a standard colorimetric method in the University Clinical Pathology Laboratory. Serum TBARS assays were performed by Metamatrix Clinical Laboratory (Duluth, Ga), which involved isolating TBARS by high-performance liquid chromatography. Results provide a measure of total serum lipid peroxidation, an indicator of cholesterol-mediated free-radical activity.

Urinary total nitrate/nitrite levels were measured by Cayman Chemical Co, Ann Arbor, Mich. A calorimetric assay method was used to measure urine nitrate/nitrite concentration, and results are reported as total nitrate/nitrite levels.

Statistical Analysis
The mean endothelial dysfunction marker levels in migraineurs and controls were obtained by ANCOVA after adjusting for age. Markers were divided on the basis of quartiles, and values corresponding to the highest or lowest quartile, depending on the marker, were used to define elevated or lower levels of markers for all study participants. Logistic-regression analysis was used to examine the association of markers with migraine and migraine type (MA, MO) compared with controls. Models were adjusted for age (continuous), body mass index (continuous), hypertension (yes, no), smoking history (yes, no), and oral contraceptive/hormone use (yes, no). Medication use was noted but not controlled for in the models, because the small numbers did not warrant inclusion in the analysis. Adjusted prevalence odds ratios (ORs) and 95% CIs were used to measure the strength of the associations between elevated or lower marker levels and the dependent variables. Significance of the ORs was examined by Wald’s $\chi^2$ statistic, and the Hosmer-Lemeshow test was used to assess the fit of the regression models. Relations between different markers and with headache frequency, age at headache onset, and duration since onset were examined by linear correlation. Applying the corrections (Benjamini-Hochberg method) for multiple-hypothesis testing did not alter the significant results of this study. All statistical analysis was performed with SAS 9.1 (SAS Institute, Inc, Cary, NC).

Results
Table 1 summarizes the subject characteristics, including demographics, headache diagnoses, and cardiovascular risk factors of the 175 female study participants (125 with migraine, 50 controls) enrolled in this study. Seventy percent of migraineurs and none of the controls were clinic patients. All eligible women seen in the clinic during the study period were invited to participate. Only 5 declined to participate, and 3 agreed to participate but were not included because a blood sample could not be obtained. The mean age of the participants was 37 years, and, by design, approximately half of the patients had MA (49%). The average number of headache days per month was 12, and 31% of the study population had ≥15 headache days per month. The majority of study participants were white, were college graduates, and had an annual household income of >$50 000. There were no differences in body mass index, hypertension, and “ever smoked” status between the groups. None of the controls currently smoked. Use of medications in migraineurs compared with that in controls was as follows: statins (12 vs 3, $P=0.331$), calcium channel blockers (12 vs 0, $P=0.015$), β-blockers (5 vs 0, $P=0.182$), and angiotensin-converting enzyme (ACE) inhibitors (6 vs 1, $P=0.356$). Forty-five percent of migraineurs were taking a migraine-preventive medication, predominantly topiramate, tricyclic antidepressants, or antihypertensives (as noted). Oral contraceptive use was higher in the control group, and we controlled for this in the analysis.

Table 2 summarizes the age-adjusted mean levels of the endothelial dysfunction markers according to migraine and MA status. Compared with controls, women with migraine had higher levels of total cholesterol, vWF activity, t-PA antigen, and hs-CRP and lower levels of total nitrate/nitrite concentration and TBARS. Approximately 29% of the migraine population and 8% of controls had vWF activity levels above the upper limit of the reference range (>150%). For hs-CRP, 66% of migraineurs and 44% of controls had levels above the upper limit of the reference range. When adjusted for age only, no significant differences in marker levels were found between migraineurs based on aura status.

Table 3 summarizes the adjusted ORs for the association of marker levels with migraine and migraine subtype. In adjusted analyses, the associations of vWF activity, hs-CRP, and nitrate/nitrite levels remained significant in the migraine group compared with controls. The associations of total
Biomarkers of endothelial activation have not been comprehensively studied in migraine, despite growing evidence of systemic endothelial dysfunction. In a young, relatively healthy cohort of women, our data support a strong relation between biomarkers of endothelial activation and migraine, including vWF activity, hs-CRP, t-PA antigen, and total nitrite/nitrate concentration. For vWF activity, hs-CRP, and total nitrite/nitrate concentration, the association was stronger for MA, the subtype most closely linked to cardiovascular disease. To our knowledge, this set of endothelium-related biomarkers, and the correlations between them, have not been previously reported in migraine.

### Oxidative Stress

Endothelial dysfunction is characterized by a reduction in the bioavailability of vasodilators and the consequent impairment of reactivity of the vasculature. It is characterized by a decrease in bradykinin-mediated endothelial nitric oxide synthesis. Our finding of decreased interictal concentrations of urinary nitric oxide stable metabolites in migraineurs compared with controls suggests more endothelial dysfunction in the migraine population. Our finding that the duration of the headache condition was correlated with nitrate/nitrite levels suggests that endothelial dysfunction may be a marker of migraine progression. The literature contains only a few studies of nitrate/nitrite concentrations in migraine. In 1 recent study of migraineurs during the interictal period, no differences in blood nitrate/nitrite concentrations were found between migraineurs and control subjects. In another, interictal nitrate/nitrite concentrations were higher than in controls, and in a third the nitric oxide metabolite concentration was lower. These studies had smaller sample sizes, used varying assay methods, included men as well as women, and lacked multivariate analysis.

### Inflammation

Vascular inflammation is a downstream result of endothelial dysfunction. It has been hypothesized to be an important part of the acute migraine process. We found that hs-CRP, a nonspecific marker of inflammation that enhances the endothelial expression of matrix metalloproteinases, was elevated in those with migraine, and the association was stronger in those with MA. There was a modest correlation with headache frequency, although causality cannot be inferred. The association of CRP with migraine has only previously been demonstrated in 2 small case-control studies and a large prospective cohort study of women >45 years of age.

### Hypercoagulability

Enhanced coagulability is a well-established consequence of endothelial dysfunction. We found elevated levels of plasma t-PA antigen in migraineurs compared with controls, reflecting reduced fibrinolysis. A case-control study of 17 persons...
with MO showed reduced t-PA levels, and we are aware of no other studies. A population-based case-control study of young (15- to 44-year-old) women, however, demonstrated that elevated plasma t-PA antigen was an independent marker of increased stroke risk.\(^\text{18}\)

vWF, widely accepted as an important plasma biomarker of endothelial dysfunction in diseased populations,\(^\text{19}\) activates platelet glycoprotein IIbIIIa receptors, causing platelet adhesion and aggregation.\(^\text{20}\) This procoagulatory protein has been associated with each major cardiovascular risk factor,\(^\text{21,22}\) as well with stroke in clinic and community-based longitudinal studies.\(^\text{23,24}\) The literature on vWF in migraine is sparse.\(^\text{25–27}\)

We had previously found that migraineurs with prior stroke had higher vWF antigen levels than did migraineurs without stroke.\(^\text{28}\) Both groups, however, had higher vWF levels than did controls. In the current study, we find mean interictal vWF activity values in migraineurs similar to those found in our 2 previous studies.\(^\text{26,27}\) Our contention that this finding of elevated vWF in migraine is indicative of endothelial dysfunction is supported by the correlations with other biomarkers. We had also found, in this same cohort, elevated vWF activity in association with the ACE deletion genotype (\(\text{DD}\)), especially when combined with the methylenetetrahydrofolate reductase thymine genotype (\(\text{TT}\)).\(^\text{29}\) These genotypes are associated with endothelial dysfunction\(^\text{29,30}\) and with MA.\(^\text{31,32}\)

This study finding of endothelial activation in migraine leads to speculation regarding the nature of the relation. Ictal studies of migraine that have documented increased vWF,\(^\text{25}\) endothelin-1,\(^\text{33}\) soluble intercellular adhesion molecule, tissue necrosis factor,\(^\text{34}\) transforming growth factor-\(\beta1\),\(^\text{35}\) and matrix metalloproteinase-9\(^\text{36}\) suggest that migraine may directly activate the endothelium. An alternative and not mutually exclusive hypothesis is that endothelial dysfunction causes migraine. Endothelin-1, a vasoconstrictor, has been shown in an in vivo rat model to be a potent inducer of cortical spreading depression, thus linking endothelial irritation to the substrate of migraine aura.\(^\text{37}\) A study in persons with migraine of recent onset demonstrated increased vascular tone and

**Table 2.** Age-Adjusted Levels of Endothelial Dysfunction Markers in Migraine

<table>
<thead>
<tr>
<th>Marker</th>
<th>Laboratory Range, Units*</th>
<th>Controls (n=50)</th>
<th>Migraineurs (n=125)</th>
<th>P Value†</th>
<th>MA (n=61)</th>
<th>MO (n=64)</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>mg/dL</td>
<td>173.0±5.2</td>
<td>187.3±3.2</td>
<td>0.0217</td>
<td>191.5±4.6</td>
<td>183.3±4.5</td>
<td>0.198</td>
</tr>
<tr>
<td>Nitrate/nitrite</td>
<td>(\mu)mol/L</td>
<td>184.5±8.6</td>
<td>26.7±5.4</td>
<td>&lt;0.0001</td>
<td>32.8±7.7</td>
<td>20.8±7.6</td>
<td>0.065</td>
</tr>
<tr>
<td>TBARS</td>
<td>(\leq)2.0 mmol/mL</td>
<td>0.84±0.05</td>
<td>0.68±0.02</td>
<td>0.001</td>
<td>0.66±0.04</td>
<td>0.695±0.04</td>
<td>0.562</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>5–15 (\mu)mol/L</td>
<td>6.46±0.2</td>
<td>6.33±0.13</td>
<td>0.409</td>
<td>6.33±0.2</td>
<td>6.34±0.2</td>
<td>0.984</td>
</tr>
<tr>
<td>vWF activity</td>
<td>50–150%</td>
<td>97.8±6.1</td>
<td>134.9±3.8</td>
<td>&lt;0.0001</td>
<td>134.5±5.4</td>
<td>135.3±5.3</td>
<td>0.921</td>
</tr>
<tr>
<td>t-PA antigen</td>
<td>&lt;14.1 ng/mL</td>
<td>4.57±0.3</td>
<td>6.00±0.2</td>
<td>0.002</td>
<td>6.11±0.4</td>
<td>5.90±0.4</td>
<td>0.680</td>
</tr>
<tr>
<td>F1 2</td>
<td>87–325 pmol/L</td>
<td>234.8±54.4</td>
<td>300.4±34.4</td>
<td>0.229</td>
<td>273.6±48.8</td>
<td>326.8±48.5</td>
<td>0.511</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>&lt;0.5 mg/L</td>
<td>1.60±0.6</td>
<td>3.96±0.4</td>
<td>0.002</td>
<td>4.56±0.58</td>
<td>3.38±0.56</td>
<td>0.202</td>
</tr>
</tbody>
</table>

Values reported in the table are age-adjusted mean±SE of marker levels.

*Laboratory reference range and measured units for markers.
†Migraineurs vs controls.
‡MA vs MO.

**Table 3.** Adjusted Logistic-Regression Analysis for Association of Markers With Migraine Compared With Controls

<table>
<thead>
<tr>
<th>Markers*</th>
<th>Controls Reference OR (95% CI)</th>
<th>P Value†</th>
<th>Migraineurs Reference OR (95% CI)</th>
<th>P Value†</th>
<th>MA Reference OR (95% CI)</th>
<th>P Value†</th>
<th>MO Reference OR (95% CI)</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>1.00</td>
<td>1.57 (0.66–3.69)</td>
<td>0.306</td>
<td>2.09 (0.82–5.32)</td>
<td>1.13 (0.43–3.03)</td>
<td>0.207</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrate/nitrite</td>
<td>1.00</td>
<td>4.65 (1.53–14.17)</td>
<td>0.008</td>
<td>6.60 (2.06–21.16)</td>
<td>3.03 (0.90–10.15)</td>
<td>0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TBARS</td>
<td>1.00</td>
<td>0.66 (0.28–1.53)</td>
<td>0.333</td>
<td>0.61 (0.23–1.63)</td>
<td>0.70 (0.27–1.86)</td>
<td>0.594</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vWF activity</td>
<td>1.00</td>
<td>5.46 (1.75–17.03)</td>
<td>0.003</td>
<td>6.51 (1.94–21.83)</td>
<td>4.59 (1.37–15.38)</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-PA antigen</td>
<td>1.00</td>
<td>1.86 (0.72–4.79)</td>
<td>0.199</td>
<td>2.51 (0.89–7.02)</td>
<td>1.32 (0.46–3.85)</td>
<td>0.154</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP</td>
<td>1.00</td>
<td>4.71 (1.48–14.97)</td>
<td>0.009</td>
<td>7.99 (2.32–27.61)</td>
<td>2.63 (0.73–9.45)</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Markers were divided on the basis of quartiles, and values corresponding to the upper quartile were used as cutoffs for total cholesterol (>206 mg/dL), TBARS (>0.9 mmol/mL), vWF activity (>147%), t-PA antigen (>5.2 ng/mL), and hs-CRP (>4.34 mg/L), and the lower quartile value was used for nitrate/nitrite (<6.14 \(\mu\)mol/L).
†‡From models adjusted for age, body mass index, hypertension, smoking history, and oral contraceptive use.
‡P value from models comparing MA, MO with controls (2 df, Wald’s \(\chi^2\) test).
decreased reactivity in the systemic circulation. This implies that endothelial dysfunction may be causally related to migraine, rather than a consequence of longstanding migraine attacks or of migraine pharmacotherapy. A number of genes associated with endothelial dysfunction appear to increase susceptibility to migraine.  

Our study has a number of strengths. All participants were interviewed and examined by the PI to ascertain migraine diagnosis or lack thereof. Collection of a detailed history allowed the data to be adjusted for confounding factors. An original aspect of our work was the use of vWF activity rather than vWF antigen. A strong correlation has been reported between levels of vWF antigen and activity, and our data on >500 migraineurs and controls support this (r=0.91, P<0.0001, authors’ unpublished data). Our study also has certain limitations. The sample size was relatively small, and certain associations may have been missed due to lack of power. The sample came from both the Headache Clinic and the general population, but with the high average monthly headache frequency, our results may not be generalizable to other populations. Despite the fact that consecutive clinic patients were invited to participate during the recruitment period and that the study was advertised to the general population, selection bias is possible. Subjects in the study discontinued taking antiplatelet agents, but other medications were not restricted, and medication use may have influenced the results. Statins, ACE inhibitors, calcium channel blockers, and β-blockers were more commonly used by the migraine group. These medications are associated with improved endothelial function and could possibly have diminished the differences in biomarkers between migraineurs and controls. We did not collect data on parity or complications of pregnancy, including pre-eclampsia. We restricted this study to women, but there are prospective data suggesting that the risk of stroke with migraine is increased in men as well. By excluding persons with diabetes and cardiovascular conditions (angina, myocardial infarction, transient ischemic attack, stroke), we may have missed important associations between migraine and endothelial dysfunction. 

In conclusion, biomarker data suggest that endothelial activation is associated with migraine, particularly MA. Further studies to determine whether these biomarkers are useful in predicting clinical and subclinical stroke risk in migraineurs are under way. An increased understanding of the role of the endothelium in migraine may provide a rationale for multiple avenues for stroke prevention in migraineurs, including use of treatments to prevent migraine, repair the vascular endothelium, and inhibit platelet aggregation and inflammation.

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