Homocysteine and Early Re-stenosis after Carotid Eversion Endarterectomy

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**Background.** Homocysteine (Hcy) appears to be involved in the development of intimal hyperplasia and arterial thrombosis. The purpose of this study was to evaluate the association of plasma Hcy with early re-stenosis following carotid eversion endarterectomy.

**Patients and methods.** Of 398 consecutive patients, 363 were included in this study. 62% of patients had symptomatic internal carotid artery (ICA) stenosis. Patients had preoperative assessment of Hcy and other well established atherosclerosis risk factors. Intraoperatively, completion angiography was performed in 2 planes. Patients had clinical, Hcy and duplex follow up at 1, 3, 18 and 36 months postoperatively.

**Results.** Complete follow up data were available for 312 patients. Five patients suffered from strokes and 2 patients died during the peri-operative period (combined stroke and death rate of 2%). Mean follow up was 26.5 months (range 17 to 36 months). Seventeen and six patients (5.5%) developed a 50–69% and >70% re-stenosis, respectively. Serum creatinine was significantly higher in patients with early re-stenosis, occlusion or stroke after CEA (P = 0.043). High grade re-stenosis, occlusion and stroke ipsilateral to the operated side (17 patients) was associated with HbA1C and creatinine (P = 0.043 and 0.046, respectively) but not Hcy.

**Conclusion.** While Hcy is a recognized independent risk factor for atherothrombosis, our study suggests that there is no association of Hcy with early re-stenosis after eversion endarterectomy.

**Keywords:** Homocysteine; Re-stenosis; Carotid endarterectomy; Local anaesthesia; Early re-stenosis.

**Introduction**

Homocysteine (Hcy) is a sulfur-containing amino acid formed during methionine metabolism.1 Elevated plasma Hcy levels have been reported to be an independent risk factor for coronary, cerebral, and peripheral arterial occlusive disease.2–5 Moreover, Hcy appears to be involved in the genesis of post-procedural intimal hyperplasia and arterial thrombosis. In *in vitro* Hcy has been shown to be a potent mitogen, leading to marked increase in vascular smooth-muscle cell proliferation6 and it has been proposed that Hcy promotes atherogenesis specifically by inducing the proliferation of vascular smooth-muscle cells.7

In an animal model of carotid endarterectomy (CEA) the degree of post-CEA intimal hyperplasia was directly related to plasma levels of Hcy. Increasing levels of plasma Hcy enhanced and accelerated smooth muscle cell response after CEA leading to increased intimal hyperplasia and luminal stenosis.8,9 Also of note, endarterectomy under general anaesthesia increases total Hcy in animals,10 an effect that was not seen in humans using local anaesthesia.11

Literature regarding the influence of Hcy on the development of early restenosis after carotid surgery in man is sparse and conflicting. So far, three clinical studies have been published, one retrospective,12 the others prospective.13,14 It was concluded in one study that restenosis is only associated with elevated Hcy after CEA at levels of >30 µmol/l.12 A second study suggested that elevated Hcy was protective against restenosis after CEA.13 In the latest study an association between elevated Hcy and re-stenosis after CEA was demonstrated.14
The aim of this study was to elucidate the role and evaluate the association of plasma Hcy with early restenosis following carotid eversion endarterectomy under local anaesthesia.

Patients and Methods

Patients

Of three hundred ninety eight consecutive patients with symptomatic and asymptomatic internal carotid artery (ICA) stenosis >70% admitted for surgery to our department, 28 refused to participate in the study. The remaining 370 were enrolled in the study during a fifteen month period. All patients were planned for routine eversion endarterectomy (EEA) of the ICA under regional anaesthesia, and underwent preoperatively duplex scanning and magnetic resonance angiography to assess the maximum lumen reduction of the ICA. Symptomatic patients with non disabling stroke had an interval of less than six weeks prior to surgery; symptomatic patients with transient hemispheric and non hemispheric symptoms were all operated on within 3 weeks of presentation. Patients were counselled and encouraged to reduce modifiable cardiovascular risk-factors and to eat a healthy diet.

Preoperative measurement of fasting total plasma Hcy, total cholesterol, LDL cholesterol, triglycerides, glycated haemoglobin (HbA1C), serum creatinine as well as assessment of hypertension, smoking habits, body mass index (BMI) and history of diabetes were done up to one week prior to surgery. All patients had an intraoperative completion angiography in two planes after reconstruction of the ICA. All patients gave written informed consent; the study was approved by the local ethics committee and in accordance to the declaration of Helsinki.

Sample handling and laboratory measurements

Blood was drawn by venipuncture of the antecubital vein and collected in standard serum and K3-EDTA tubes (Vacuette EDTA Tubes, Greiner Bio-One, Kremsmünster, Austria). Blood samples were centrifuged (10 minutes at 2,000 G) and plasma for Hcy analysis was decanted within 15 minutes after sampling and stored at minus 80 degrees Celsius until analysis. Hcy measurements were performed on an Abbott AxSYM Plus Immunology analyzer (Abbott Diagnostics, Abbott Park, IL, USA) using Abbott AxSYM Hcy reagents according to manufacturer’s instructions. The sensitivity of the AxSYM homocysteine assay was assessed to be 0.8 μmol/l by replicate analyses of the zero-level calibrator. Sensitivity was defined as the concentration at two standard deviations from the mean and represents the lowest measurable concentration of Hcy that can be distinguished from zero. The coefficient of variation (CV) resulting from intra-assay precision experiments was between 4.5 and 1.4% at concentrations ranging from 7.6 to 28.2 μmol/l. Evaluating inter-assay imprecision in the same concentration range the CV was between 2.0 and 5.1%.

Analysis of total cholesterol, HDL cholesterol, triglycerides and creatinine in serum was performed on a Hitachi 917 analyzer (Roche Diagnostics, Mannheim, Germany), HbA1C in K3-EDTA whole blood was analyzed on a Cobas Integra 700 analyzer (Roche Diagnostics, Basel, Switzerland) according to manufacturer’s instructions.

Follow up

Patients included in the study had clinical and morphological (Duplex) follow up at 1, 3, 18 and 36 months post operation. A second measurement of Hcy was performed between 18 and 36 months postoperatively. Only patients with complete follow up were evaluated. The progression of lumen stenosis from the first to the second postoperative measurement was assessed for all patients. Endpoints for cases were defined as degrees of restenosis of 50%–69% (low grade restenosis) and stenosis 70% to 99% (high grade restenosis), recurrence of symptoms, stroke and stroke related death ipsilateral to the operated ICA during follow up.

Duplex criteria

Duplex grading of ICA stenosis was according to previously published criteria. Peak systolic velocities (PSV) within the ICA of 150 to 249 mm/sec and the ratio of PSV of the ICA and common carotid artery (CCA) of 2.0 to 3.9 were quantified as 50% to 69% stenosis. PSV within the ICA greater 250 mm/sec and the ratio of PSV of the ICA and CCA of greater 4 were quantified as 70% to 99% stenosis.

Statistical analysis

The study was designed as a case-control analysis following a prospective data collection. After final completion of data collection, patients were allotted to the case group or control group based on the results of duplex grading of ICA. A patient was regarded as a case if during follow up a re-stenosis of ≥50%,
occlusion, stroke or stroke related death ipsilateral to the operated ICA occurred. All other patients were regarded as controls.

Results were analyzed using a software package (Epi-Info 2002 software package, Centers for Disease Control and Prevention, Atlanta, GA, USA).

Continuous variables were shown as mean ± SD together with minimum and maximum values (range); means were compared with a Student’s t-test for unpaired variables. Discrete variables were expressed as numbers (percentages) and compared by chi-square test or by Fisher’s exact test, where appropriate. Because our null hypothesis stated that Hcy levels ≥12 μmol/l are associated with early re-stenosis or occlusion after CEA, one-sided P values were computed. P values of ≤ .05 were considered significant.

Results

Of 370 patients prospectively enrolled in the study, 1 had a PTFE interposition and 6 were operated under general anaesthesia. Thus 363 were included in the study. 198 were male, 165 female and their age ranged from 40 to 92 years (mean 71 ± 9). 139 patients (38%) had asymptomatic ICA stenosis, 151 patients (42%) had transient ischemic episodes and 73 patients (20%) were operated on for non-disabling stroke. Five patients suffered from perioperative stroke (one associated with ICA occlusion), 2 patients died perioperatively (one stroke related death), accounting for a perioperative stroke and death rate of 2%. Of the Five patients with stroke 3 were lost to follow up. Complete follow up data were available for 312 of 363 patients (86%) enrolled in the study (Tables 1 and 2).

Two hundred seventy eight patients had an uneventful follow up without reaching any study endpoint. One hundred and sixty four patients (59%) were male, 114 (41%) female, their mean age was 71 ± 8 years (range 40 to 92). Mean follow up was 26 ± 6 months (range 18 to 38 months).

Thirty four patients fulfilled criteria of the endpoint (11%), 17 patients (5.5%) had a re-stenosis between 50 and 69%, 6 patients (2%) developed a re-stenosis of >70%, 6 patients (2%) died of stroke ipsilateral to the operated side and 5 patients (1.6%) had an asymptomatic occlusion of the operated ICA. Twenty four (70%) were male, 10 (30%) female, their mean age was 71 ± 8 years (range 56 to 84). Mean follow up was 26 ± 5 months (range 17 to 36 months).

Of all patients, 187 patients (60%) had Hcy levels ≥12 μmol/l, 165 patients with uneventful follow up (60%) and 23 patients reaching the endpoint (67%;11 patients with low grade re-stenosis, 12 patients with high grade re-stenosis, occlusion or stroke). There was no statistically significant difference between Hcy in these groups (no restenosis compared to all cases P = 0.340; no restenosis compared to patients with low grade re-stenosis P = 0.662; no restenosis compared to patients with high grade re-stenosis, occlusion or stroke P = 0.358; and low grade restenosis compared to high grade restenosis, occlusion or stroke P = 0.713).

Hcy in general and in both groups did not change during follow up. Preoperative Hcy for all patients compared to follow up was 14.20 ± 7.05 μmol/l (5.96–89.23 μmol/l) and 14.19 ± 6.98 μmol/l (5.90–81.11 μmol/l). Preoperative Hcy for cases was 15.03 ± 5.08 μmol/l (8.23–30.23 μmol/l) prior to operation and 15.00 ± 5.08 μmol/l (8.13–34.09 μmol/l) during follow-up. Preoperative Hcy for all controls compared to follow up was 14.09 ± 7.26 μmol/l (5.96–89.23) and 14.12 ± 7.20 μmol/l (5.73–86.00 μmol/l).

Serum creatinine was significantly higher in patients with stenosis >70% or occlusion

Table 1. Continuous variables

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cases n = 34</th>
<th>Controls n = 278</th>
<th>All n = 312</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocystein μmol/l</td>
<td>Mean ± SD (range)</td>
<td>Mean ± SD (range)</td>
<td>Mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td>15.03 ± 5.08 (8.23–30.23)</td>
<td>14.09 ± 7.26 (5.96–89.23)</td>
<td>14.20 ± 7.05 (5.96–89.23)</td>
<td>0.233</td>
<td></td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.97 ± 4.37 (16.44–34.50)</td>
<td>26.14 ± 3.66 (16.16–38.97)</td>
<td>26.12 ± 3.74 (16.16–38.97)</td>
<td>0.399</td>
</tr>
<tr>
<td>HbA1C %</td>
<td>6.15 ± 0.94 (4.80–9.00)</td>
<td>6.02 ± 0.87 (4.80–10.40)</td>
<td>6.03 ± 0.88 (4.80–10.40)</td>
<td>0.196</td>
</tr>
<tr>
<td>Creatinine μmol/l</td>
<td>105.2 ± 27.4 (44.2–185.6)</td>
<td>96.4 ± 27.4 (44.2–256.4)</td>
<td>97.2 ± 30.1 (44.2–256.4)</td>
<td>0.043*</td>
</tr>
<tr>
<td>Cholesterol mmol/l</td>
<td>5.14 ± 1.02 (3.78–8.35)</td>
<td>5.21 ± 1.05 (2.59–10.32)</td>
<td>5.20 ± 1.05 (2.59–10.32)</td>
<td>0.351</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.35 ± 0.38 (0.75–2.43)</td>
<td>1.37 ± 0.41 (0.41–3.78)</td>
<td>1.37 ± 0.41 (0.41–3.78)</td>
<td>0.389</td>
</tr>
<tr>
<td>LDL mmol/l</td>
<td>3.03 ± 0.94 (1.78–6.36)</td>
<td>3.03 ± 0.95 (0.96–7.76)</td>
<td>3.03 ± 0.95 (0.96–7.76)</td>
<td>0.493</td>
</tr>
<tr>
<td>Triglycerides mmol/l</td>
<td>1.68 ± 0.87 (0.58–5.31)</td>
<td>1.78 ± 0.97 (0.43–7.81)</td>
<td>1.77 ± 0.96 (0.43–7.81)</td>
<td>0.285</td>
</tr>
<tr>
<td>Uric acid μmol/l</td>
<td>379.0 ± 102.3 (178.5–701.9)</td>
<td>356.9 ± 97.6 (119.0–832.8)</td>
<td>359.3 ± 98.2 (119.0–832.8)</td>
<td>0.114</td>
</tr>
<tr>
<td>Follow up (months)</td>
<td>26 ± 5 (17–36)</td>
<td>26 ± 6 (18–48)</td>
<td>26 ± 6 (17–48)</td>
<td>0.327</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71 ± 8 (56–84)</td>
<td>71 ± 9 (40–92)</td>
<td>71 ± 9 (40–92)</td>
<td>0.478</td>
</tr>
</tbody>
</table>

BMI: body mass index; HDL: high density lipoprotein; LDL: low density lipoprotein.
developed during follow-up compared to controls (two tailed t-test, \( p = 0.043 \)). All other parameters, including Hcy, did not differ statistically significantly.

Comparing only patients with high grade restenosis, occlusion and stroke ipsilateral to the operated side (17 patients), HbA1C and creatinine, but no Hcy, were significantly elevated compared to patients without restenosis (two tailed t-test, \( p = 0.043 \) and 0.046, respectively).

Discussion

Hcy has been described as an independent risk factor for recurrent stroke and major vascular events. Reduction of Hcy levels by supplementation of folic acid, pyridoxine and cobalamin, however, had no effect on recurrent stroke or myocardial infarction. Yet, reduction of Hcy after endovascular intervention of coronary arteries significantly reduced the incidence of major vascular events. This effect was explained by a reduction of the prothrombotic propensities of Hcy and its stimulating effects on proliferation of vascular smooth muscle cells and thus intima-media thickening.

To date, the effect of Hcy on early restenosis after CEA has not been answered unequivocally. Prospective studies have yielded contradictory results and included very small sample sizes of 86 and 68 patients, respectively. In our current study, 34 patients (11% of all with complete follow up) reached a combined endpoint of re-stenosis, occlusion, stroke and stroke associated death ipsilateral to the operated ICA during a mean follow up of 26 months. Six patients (2%) had a high grade restenosis (>70%), one being symptomatic. This finding is in keeping with large randomized trials for restenosis after endarterectomy of the ICA.

As all patients had intraoperative angiography and Duplex scanning one to three months postoperatively, residual stenoses were excluded as confounders.

In total, 60% of our patient population had an Hcy of \( \geq 12 \) \( \mu \text{mol/l} \), a threshold reported to bear a 2.2 fold relative risk of vascular disease. There was no statistically significant difference in Hcy levels between cases and controls. Thus, it appears that atherothrombotic effects of Hcy alone do not play a prominent role in early re-stenosis or occlusion after endarterectomy of the ICA under local anesthesia.

There was also no association of other traditional risk factors with restenosis. Cases had, however, a significantly elevated serum creatinine compared to patients without re-stenosis. These findings corroborate previous reports that re-stenosis after carotid endarterectomy possibly differs from mechanisms of atherosclerosis leading to lumen reduction or re-stenosis after endovascular interventions with and without stent.

Importantly, when comparing only patients with high grade re-stenosis (6 patients), asymptomatic occlusion (5 patients) and stroke ipsilateral to the operated side (6 patients), HbA1C and creatinine, but not Hcy, were significantly elevated compared to patients without re-stenosis (two tailed t-test, \( p = 0.043 \) and 0.046, respectively). This is in keeping with observations indicating increased risk of cardiovascular and cerebrovascular events in diabetic patients with elevated Hcy.

The molecular mechanisms of the detrimental effects of Hcy in diabetics are not fully understood. These effects may be due to an accelerated glucose induced oxidative stress. Thus, the potentiation of diabetic angiopathy by Hcy could be mediated by impairment of NO formation and overproduction of superoxide.

Our study has one limitation. Based on previous reports, our sample size calculation was performed assuming a 20% difference in Hcy levels between both groups of patients. To be able to detect a 20% difference at a power of 80% (two-tailed significance 0.05), we calculated that a total of 297 patients, 27 in the case group, and 270 in the control group, would be necessary. Therefore, our study is well powered to

Table 2. Categorical variables

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Cases ( n = 34 ) (%)</th>
<th>Controls ( n = 278 ) (%)</th>
<th>All ( n = 312 ) (%)</th>
<th>OR</th>
<th>95% CI</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>24 (71)</td>
<td>164 (59)</td>
<td>188 (60)</td>
<td>1.67</td>
<td>0.73–3.90</td>
<td>0.192</td>
</tr>
<tr>
<td>Female</td>
<td>10 (29)</td>
<td>114 (41)</td>
<td>124 (40)</td>
<td>0.60</td>
<td>0.26–1.37</td>
<td>0.192</td>
</tr>
<tr>
<td>CAD; asymptomatic</td>
<td>16 (47)</td>
<td>117 (42)</td>
<td>133 (43)</td>
<td>1.22</td>
<td>0.57–2.64</td>
<td>0.579</td>
</tr>
<tr>
<td>CAD; TIA</td>
<td>12 (35)</td>
<td>104 (37)</td>
<td>116 (37)</td>
<td>0.91</td>
<td>0.41–2.03</td>
<td>0.809</td>
</tr>
<tr>
<td>CAD; stroke</td>
<td>6 (18)</td>
<td>57 (21)</td>
<td>63 (20)</td>
<td>0.83</td>
<td>0.27–2.18</td>
<td>0.695</td>
</tr>
<tr>
<td>Current smoker</td>
<td>9 (26)</td>
<td>79 (28)</td>
<td>88 (28)</td>
<td>0.91</td>
<td>0.37–2.15</td>
<td>0.811</td>
</tr>
<tr>
<td>Diabetic</td>
<td>12 (35)</td>
<td>73 (25)</td>
<td>85 (27)</td>
<td>1.53</td>
<td>0.67–3.44</td>
<td>0.263</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29 (85)</td>
<td>227 (82)</td>
<td>256 (82)</td>
<td>1.30</td>
<td>0.45–404</td>
<td>0.601</td>
</tr>
</tbody>
</table>

CAD: carotid artery disease; TIA: transient ischaemic attack.

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detect a 20% difference, but not lesser differences. Indeed, the difference between the Hcy means of our cases and controls groups was only 7%. To be able to detect a difference of 7% between two groups, we would have had to encompass a total of 2387 patients, 217 in the cases group and 2170 in the controls group.

In conclusion, the results of this study do not demonstrate a significant difference of plasma Hcy in patients with early re-stenosis, occlusion or stroke after carotid endarterectomy compared to those without. While Hcy is a recognized independent risk factor for atherothrombosis, our study suggests that there is no association of Hcy with early restenosis after endarterectomy.

References


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