Hyperhomocysteinemia as an Independent Risk Factor for Cardioembolic Stroke in the Turkish Population

Nida Tascilar,1 Sureyya Ekem,1 Esra Aciman,1 Handan Ankarali,2 Gorkem Mungan,3 Banu Ozen1 and Aysun Unal1

1Department of Neurology, Zonguldak Karaelmas University Medical Faculty, Zonguldak, Turkey
2Department of Biostatistics, Zonguldak Karaelmas University Medical Faculty, Zonguldak, Turkey
3Department of Biochemistry, Zonguldak Karaelmas University Medical Faculty, Zonguldak, Turkey

Homocysteine, a sulfur-containing amino acid, is an intermediate during the conversion of methionine to cysteine. Homocysteine can cause vascular injury and atherosclerotic plaque instability. In addition, homocysteine may be directly correlated with hyperlipidemia and lipoprotein(a) and inversely with high-density lipoprotein cholesterol. However, the results regarding the association of homocysteine level with subtypes of stroke and traditional risk factors for stroke have been inconsistent, perhaps due to ethnic differences. The aim of this study was to evaluate the role of serum homocysteine levels in Turkish patients diagnosed with atherosclerotic stroke and those with cardioembolic stroke. We measured homocysteine levels, traditional risk factors for stroke (hypertension, diabetes mellitus, and smoking) and lipoprotein(a) levels in 103 patients with large-vessel atherosclerotic stroke, 37 patients with cardioembolic stroke, and 37 controls with normal cranial magnetic resonance imaging. Only hypertension was found to be a risk factor in all patient groups ($p = 0.001$). Hyperhomocysteinemia (homocysteine level $\geq 15.90 \mu mol/L$) was more common in patients with large-vessel atherosclerotic stroke and cardioembolic stroke ($p = 0.0435$ and $p = 0.007$, respectively); nevertheless, it was found to be a risk factor only in patients with cardioembolic stroke ($p = 0.023$; odds ratio (OR): 5.745). Furthermore, in the patients with large-vessel atherosclerotic stroke, hyperhomocysteinemia was positively correlated with the lipoprotein(a) level ($r = 0.227$, $p = 0.035$). In conclusion, hyperhomocysteinemia is common in patients with large-vessel atherosclerotic stroke and cardioembolic stroke. More importantly, hyperhomocysteinemia is an independent risk factor only for cardioembolic stroke in the Turkish population.

Stroke is the third leading cause of death and disability in adults even in industrialized countries (Biller and Love 2004). Some of the important risk factors for ischemic stroke include older age, male gender, arterial hypertension (HT), diabetes mellitus (DM), dyslipidemia, cigarette smoking, excessive alcohol intake, and high body mass index (BMI) (Biller and Love 2004). However, it has become increasingly apparent that apart from the traditional risk factors associated with stroke, there are several newer independent modifiable risk factors, such as hyperhomocysteinemia (Okubadejo et al. 2008). Homocysteine is an intermediate for the conversion of methionine to cysteine (Choy et al. 2000). It is metabolized via three pathways: 1) catabolized to cysteine via the $B_6$-dependent enzyme cystathionine beta-synthase, 2) remethylated to methionine via the folic acid and $B_{12}$-dependent enzyme methionine synthase (in this pathway, vitamin $B_{12}$ is an essential cofactor for methionine synthase, 5′-methyltetrahydrofolate is the methyl group donor and the methylenetetrahydrofolate reductase (MTHFR) functions as a catalyst in the reduction of 5′-10-methyltetrahydrofolate to 5′-methyltetrahydrofolate), and 3) remethylated via the enzyme betaine-homocysteine methyltransferase (Kittner et al. 1999; Zhang and Dai 2001). Hyperhomocysteinemia could be due to enzymatic defects (cystathionine beta-synthase, MTHFR, methionine synthase) or vitamin deficiencies (vitamin $B_{12}$, vitamin $B_6$, folate) in one or more of the homocysteine metabolizing pathways (the remethylation and/or the transulfuration) (Refsum et al. 1998; Zhang and Dai 2001; Virtanen et al. 2005; Okubadejo et al. 2008). McCully, in 1969, was the first to suggest high levels of homocysteine as a cause of atherosclerosis (McCully 1969). Since then, studies examining the relationship of hyperhomocysteinemia with atherosclerotic vascular diseases (Hankey and Eikelboom 1999; Ueland et al. 2000), including ischemic stroke (Hankey and Eikelboom 1999), have been designed. It was
suggested that hyperhomocysteinemia could be one of the unnoticed risk factors in patients with atherosclerotic stroke (AS) and embolic stroke (ES), since control of the known risk factors for atherothromboembolism failed to prevent AS (Hankey and Warlow 1999; Yokote et al. 2007) and oral anticoagulant therapy in the treatment of ES (Hart et al. 1999) failed to prevent recurrent stroke attacks and other serious vascular events (Hankey and Warlow 1999; Poli et al. 2005). Some studies have shown that high plasma homocysteine was strongly associated with large-artery atherosclerosis (Eikelboom et al. 2000; Shimizu et al. 2002), but others failed to confirm this (Evers et al. 1997; Eikelboom et al. 2000; Yokote et al. 2007). This kind of discrepancy was also found in cardioembolic stroke (CES). While some investigators showed a relationship between moderate hyperhomocysteinemia and the occurrence of stroke in patients with non-avalval atrial fibrillation (AF) (Friedman 2001; Cingozbay et al. 2002), others were unable to determine any such relationship between hyperhomocysteinemia and CES (Eikelboom et al. 2000). These conflicting findings may be attributed to the use of different stroke classifications, specific exclusion criteria, or to the selection criteria of the controls. It was previously stated that elevated plasma homocysteine could correlate with increased levels of cholesterol, lipoprotein (a) [Lp(a)] and decreased levels of high-density lipoprotein cholesterol (HDL-c) (Choy et al. 2000; Dhamija et al. 2009). It was thus suggested that the relationship between hyperhomocysteinemia and atherogenesis development could depend on its ability to produce higher cholesterol since hyperlipidemia is one of the most important risk factors in atherosclerosis and the plasma level of HDL-c, which serves as a “scavenger” for cholesterol, is inversely related to the occurrence of hyperlipidemia (Choy et al. 2000).

In view of the above, we attempted in this study to evaluate: 1) the relationship between homocysteine levels and stroke due to large-vessel AS (LVAS) and CES; 2) the relationship of homocysteine with traditional risk factors; and 3) the relationship of homocysteine with Lp(a) (which has an atherogenic effect) (Marcovina and Koschinsky 2003) and HDL-c (which has an antiatherogenic effect) (Choy et al. 2000).

**Subjects and Methods**

**Patients**

Patients with stroke who were admitted to the Neurology Department of our hospital between January 1, 2003 and January 1, 2009 were evaluated retrospectively. LVAS and CES were classified according to the TOAST criteria (Adams et al. 1993). Diagnosis of stroke was based on the results of strict neurological examination and brain computed tomography (BCT) or cranial magnetic resonance (CMR) imaging. Patients were excluded in the presence of: strokes of undetermined type (due to lack of imaging or any other diagnostic procedures to determine stroke type, such as echocardiography, Doppler ultrasonography of carotid and/or vertebral arteries, etc.); other subtypes of stroke (small vessel AS, transient ischemic attack, intracerebral hemorrhage, subarachnoid hemorrhage, arterial dissection, embolic brain infarction due to valvular-AF, brain tumors, and cerebrovascular malformation); any other known neurologic diseases (Alzheimer’s dementia, Parkinson’s disease, etc.); severe systemic diseases (chronic renal failure, collagen vascular diseases, endocrine [including hypothyroidism and hyperthyroidism] and liver and metabolic disease (except for DM); inflammation (septicemia, tuberculosis, etc.); malignant diseases; and use of anticonvulsants, multivitamins, methotrexate or nitrous oxide (which influence homocysteine level) (Pezzini et al. 2006; Censori et al. 2007) at the time of the homocysteine assay. According to the above-mentioned exclusion criteria, only 140 patients with 1 of 2 subtypes of stroke (LVAS and CES), whose homocysteine concentrations were determined 24 hours after stroke, were included into the study.

Complete medical history, including HT, DM, ischemic heart disease, AF, hyperlipidemia, smoking, alcohol intake, drug treatments (antihypertensives, statins, multivitamins, etc.) were obtained from the patients’ medical records. HT and DM were defined according to the criteria of the World Health Organization (1999). Smoking status was determined according to the patients’ medical records. HT and DM were defined according to the literature (Poli et al. 2005; Pezzini et al. 2006). Smoking (variables known to influence their levels) (Pulkkinen et al. 2000). While studying Lp(a) level and its relationship with homocysteine, patients and controls were analyzed after adjusting for age, sex, and smoking (variables known to influence their levels) (Pulkkinen et al. 2000). Homocysteine, vitamin B12, and folate levels were measured by chemiluminescent competitive immunoassay on DPC Immulite 2000 analyzer (Bio DPC; Los Angeles, USA) using commercial kits. The reference intervals were 5-12 µmol/L for homocysteine, 160-800 pg/ml for vitamin B12, and 3.0-17.0 ng/ml for folic acid.

The intra- and interassay coefficients of variation (CVs) were below 5.0%, 5.0% and 1.5% for homocysteine, vitamin B12, and folate, respectively. Analytical sensitivity for each was determined as 0.5 µmol/L, 50 pg/ml and 0.3 ng/ml, respectively.
**Statistical Analysis**

Descriptive values were calculated as mean ± s.d. and frequency (number and percent). Kolmogorov-Smirnov test was used for normality test of continuous variables. In statistical analysis, log transformed values of homocysteine and Lp(a) were used. First, one-way ANOVA was used to determine differences between groups (control, LvAS and CES) with regard to continuous variables, and Pearson chi-square test was used for categorical variables. According to the results of these univariate analyses, multiple models were then conducted. Covariance analysis was used for differences between groups (control and LvAS) with regard to HDL. In these models, smoking, BMI and age were selected as covariates. Covariance analysis was used for comparison of groups (control, LvAS, CES). In this model, age, sex and presence of smoking, HT, DM, and hyperlipidemia were included as covariates. Multiple multinomial logistic regression model was used for determining the relation between groups (control, LvAS and CES) and HT, sex, quartiles of homocysteine, and presence of hyperlipidemia. Type I error was accepted as 0.05 and SPSS (ver. 11.5) program was used for all statistical calculations.

**Results**

**Characteristics of Cases**

Of the 140 patients, 103 were subtyped as LvAS and 37 as CES. Spontaneous echo contrast (SEC) or thrombus was detected in 7 patients with CES by echocardiography. Baseline characteristics of the patients and controls are shown in Table 1. Controls were younger than the patient groups, and the CES group was older than the LvAS group, and the differences were statistically significant \( p < 0.0001 \) for both. A statistically significant difference was also found between the LvAS and control group according to gender \( p = 0.002 \). With respect to the traditional risk factors, there was no statistically significant difference between patient and control groups regarding smoking; HT was found to be the only statistically significant risk factor in all patient groups \( p = 0.001 \); DM was determined as a risk factor only in the LvAS group \( p = 0.043 \); and hyperlipidemia was not significant between patient and control groups, but was more common in the LvAS than the CES group.

### Table 1. Characteristics of the patients and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group ((n = 37))</th>
<th>LvAS ((n = 103))</th>
<th>CES ((n = 37))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>37-71</td>
<td>30-90</td>
<td>42-88</td>
</tr>
<tr>
<td>mean (s.d.)</td>
<td>53 (7.45)</td>
<td>61.19 (14.20)</td>
<td>73.35 (10.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14/37) (37.8)</td>
<td>(70/103) (68)**</td>
</tr>
<tr>
<td>Sex (male) (%)</td>
<td>14/37 (37.8)</td>
<td>70/103 (68)**</td>
<td>17/37 (45.9)</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>10/30 (30.3)</td>
<td>29/95 (30.5)</td>
<td>4/37 (16.7)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>16/36 (44.4)</td>
<td>73/101 (72.3)**</td>
<td>30/37 (81.1)**</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>6/36 (16.7)</td>
<td>40/101 (39.6)*</td>
<td>12/37 (32.4)</td>
</tr>
<tr>
<td>Hyperlipidemia (%)</td>
<td>21/37 (56.8)</td>
<td>69/103 (67)#</td>
<td>12/37 (32.4)</td>
</tr>
<tr>
<td>Folate, mean (s.d.) (nmol/l)</td>
<td>8.79 (2.74)</td>
<td>8.67 (3.80)</td>
<td>9.29 (3.89)</td>
</tr>
<tr>
<td>Vitamin B(_{12}), mean (s.d.) (pmol/l)</td>
<td>401.23 (350.98)</td>
<td>406.44 (383.12)</td>
<td>416.37 (337.98)</td>
</tr>
</tbody>
</table>

LvAS, large-vessel atherosclerotic stroke; CES, cardioembolic stroke; s.d., standard deviation.

Comparison of patient groups and control group: \(^*: p < 0.05\); \(^**: p < 0.01\); \(^: p < 0.001\)

Comparison of LvAS and CES: \(^: p < 0.01\); \(^: p < 0.001\)

Numbers of the patients and controls were varied depending on the parameters, because some data, such as smoking habits, were not available.

### Table 2. Homocysteine levels in the patient and control groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Homocysteine levels Mean ± s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (37)</td>
<td>10.98 ± 2.91</td>
</tr>
<tr>
<td>Patient Groups (140)</td>
<td></td>
</tr>
<tr>
<td>LvAS (103)</td>
<td>13.94 ± 6.56</td>
</tr>
<tr>
<td>CES (37)</td>
<td>14.96 ± 5.94*</td>
</tr>
<tr>
<td>Non-valvular AF (+) (34)</td>
<td>14.80 ± 6.15*</td>
</tr>
<tr>
<td>SEC or thrombus in ECHO (7)</td>
<td>14.90 ± 5.06</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; LvAS, large-vessel atherosclerotic stroke; CES, cardioembolic stroke; SEC, spontaneous echo contrast; ECHO, echocardiography.

* Mean homocysteine level was significantly higher in patients with CES and CES with non-valvular AF than in controls \( p = 0.011 \).
Although homocysteine levels were negatively correlated with folate [in control \( r = -0.519, p = 0.002 \) and LvAS \( r = -0.330, p = 0.001 \) groups] and vitamin B\(_{12}\) [in LvAS \( r = -0.294, p = 0.004 \) group], folate and vitamin B\(_{12}\) levels were not significantly different between patient and control groups \( p > 0.05 \). No association of vitamin status with stroke was determined.

By covariance analysis, it was found that HDL-c, which is an antiatherogenic cholesterol, was not affected by gender, BMI, or smoking. Hence, no adjustments were made for this variable. In the LvAS group, although HDL-c level was lower than in the control group, the difference was not statistically significant [mean ± SE: 45.87 ± 2.90 mg/dl and 40.68 ± 1.85 mg/dl, respectively \( p > 0.05 \)].

**Homocysteine Profile According To:**

a. *Stroke subtypes:* Mean homocysteine levels were not significantly different between LvAS and control groups \( p > 0.05 \), but homocysteine level was significantly higher in patients with CES and CES with non-valvular AF than in controls \( p = 0.011 \) (Table 2).

When patients and controls were analyzed with respect to total homocysteine distribution by quartile (quartile 1: 4.00 - 9.20 \( \mu \)mol/L; quartile 2: 9.21 - 12.40 \( \mu \)mol/L; quartile 3: 12.70 - 15.80 \( \mu \)mol/L; quartile 4: 15.90 - 42.80 \( \mu \)mol/L). Higher percentages of patients in large-vessel atherosclerotic stroke (LvAS) and cardioembolic stroke (CES) groups had total homocysteine level in the fourth quartile compared to control group \(( p = 0.0435 \text{ and } p = 0.007, \text{ respectively})\).

By covariance analysis, it was found that HDL-c, which is an antiatherogenic cholesterol, was not affected by gender, BMI, or smoking. Hence, no adjustments were made for this variable. In the LvAS group, although HDL-c level was lower than in the control group, the difference was not statistically significant [mean ± SE: 45.87 ± 2.90 mg/dl and 40.68 ± 1.85 mg/dl, respectively \( p > 0.05 \)].

**Homocysteine Profile According To:**

a. *Stroke subtypes:* Mean homocysteine levels were not significantly different between LvAS and control groups \( p > 0.05 \), but homocysteine level was significantly higher in patients with CES and CES with non-valvular AF than in controls \( p = 0.011 \) (Table 2).

When patients and controls were analyzed with respect to total homocysteine distribution by quartile (quartile 1: 4.00 - 9.20 \( \mu \)mol/L; quartile 2: 9.21 - 12.40 \( \mu \)mol/L; quartile 3: 12.70 - 15.80 \( \mu \)mol/L; quartile 4: 15.90 - 42.80 \( \mu \)mol/L), significantly higher percentages of patients in the LvAS and CES groups had total homocysteine level in the fourth quartile compared to controls \(( p = 0.0435 \text{ and } p = 0.007, \text{ respectively})\) (Fig. 1, Table 3). However, when stroke risk factors were analyzed by multiple multinomial logistic regression model, it was found that total homocysteine distribution in
Hyperhomocysteinemia in Stroke

297

the fourth quartile increased the risk of CES 5.7-fold ($p = 0.023$), HT increased the risk of CES 6.5-fold ($p = 0.001$) and of LVAS 3.7-fold ($p = 0.003$), and male gender increased the risk of LVAS 3.9-fold ($p = 0.002$) (Table 4).

b. Age, gender, HT, DM, cigarette smoking, and hyperlipidemia: Although controls were younger than patients, homocysteine levels did not differ according to age in the patient and control groups ($p > 0.05$). Homocysteine levels were also analyzed with respect to gender, smoking and presence of HT, DM and hyperlipidemia, and no statistically significant differences were determined between the groups ($p > 0.05$).

c. HDL-c and Lp(a): While homocysteine was negatively correlated with HDL-c ($r = -0.375, p = 0.022$) in the control group, it was positively correlated with Lp(a) ($r = 0.227, p = 0.035$) in the LVAS group.

Discussion

Hyperhomocysteinemia is suggested to be one of the newer modifiable risk factors for CES and ischaemic stroke in some populations (Ay et al. 2003; Okubadejo et al. 2008). In our study, mean homocysteine level was higher only in patients with CES (especially in those with AF) compared to controls. However, when analysis was done with respect to total homocysteine distribution quartiles, more patients with LVAS and CES had total homocysteine levels in the fourth quartile compared to controls. It could thus be suggested that hyperhomocysteinemia was significantly more common in patients with LVAS and CES; nevertheless, it was found to be a risk factor only in patients with CES.

Results from epidemiologic studies on the relation between homocysteine and stroke widely conflict, which may be attributed to the different study designs or ethnic groups (Verhoef et al. 1994; Bots et al. 1999; Li et al. 2003; Ntaios et al. 2008). While some studies suggest a positive association between homocysteine and stroke, such as in the Chinese population, British males, Italians, and Netherlanders (Perry et al. 1995; Graham et al. 1997; Giles et al. 1998; Bots et al. 1999; Madonna et al. 2002; Li et al. 2003), others did not confirm this, such as in Finnish, Nigerians and in a small-sized study in Netherlander patients (Alfthan et al. 1994; Verhoef et al. 1994; Sacco et al. 2004). Some authors showed that elevated homocysteine promoted large-vessel atherosclerosis (Yoo et al. 1998; McQuillan et al. 1999; Spence et al. 1999; Pezzini et al. 2006; Yokote et al. 2007). We also showed in our study that significantly higher percentages of patients in the LVAS had hyperhomocysteinemia ($p = 0.0435$).

In some studies, it was stated that the association between homocysteine and ischemic stroke is codependent on lifestyle and environmental and vascular risk factors (Piyathilake et al. 1994; Nygard et al. 1998; O’Callaghan et al. 2002), such as high blood pressure, smoking history, old age, gender, DM, elevated cholesterol level-hyperlipidemia, and lack of exercise (Yoo et al. 1998; Choy et al. 2000; Li et al. 2003; Refsum et al. 2004; Loffredo et al. 2005; Virtanen et al. 2005; Pezzini et al. 2006; Refsum et al. 2006; Censori et al. 2007; Youssef et al. 2007; Ntaios et al. 2008), while others have shown that high and moderately elevated homocysteine levels are potentially modifiable risk factors for stroke in all age groups, independent of the effect of smoking, cholesterol and blood pressure (Perry et al. 1995;
Eikelboom et al. 2000; Okubadejo et al. 2008). Our results show that homocysteine levels are independent of the effects of traditional risk factors, age and gender.

Furthermore, it was shown in some studies that decrease in homocysteine level could cause a decrease in Lp(a) (Naruszewicz et al. 2001; Dhamija et al. 2009) and an increase in HDL-c level (Glueck et al. 1995) and thereby promoting atherogenesis development (Choy et al. 2000). In our study, similar findings were observed in some groups. An increase in homocysteine level corresponded to a decrease in the level of HDL-c in the control group and an increase in Lp(a) in LvAS patients (p < 0.05). Nevertheless, no association was found between hyperlipidemia and homocysteine level in any group.

As for CES, it was recently reported that hyperhomocysteinemia is a possible target to prevent thrombotic events in patients with non-valvular AF (Friedman 2001; Cingozbay et al. 2002; Ay et al. 2003). In the study of Ay et al., they suggested that hyperhomocysteinemia may favor clot formation in the left atrium of patients with non-valvular AF and acute stroke (Ay et al. 2003). An association of hyperhomocysteinemia with the occurrence of stroke in patients with non-valvular AF, whether or not they were on oral anticoagulant therapy, has also been shown (Loffredo et al. 2005; Poli et al. 2005). In our population, we showed that hyperhomocysteinemia was associated with CES (p = 0.007) and it increased the risk of CES 5.7 - fold (p = 0.023).

Some authors proposed that homocysteine could cause not only vascular injury by endothelial toxicity and increased proliferation of smooth muscle cells, free radical-mediated cellular damage, and platelet activation (Choy et al. 2000; Spence 2006; Censori et al. 2007; Okubadejo et al. 2008), but also plaque instability, which in turn causes microemboli (Yokote et al. 2007) and coagulation (Freyburger et al. 1997). These could be among the many mechanisms underlying the development of LvAS and/or CES stroke in our population.

There are a few limitations to the study. First, due to its retrospective nature, there is a probability that the history of drug use ascertained is imprecise. Hence, we could not evaluate the relationship of homocysteine with LDL-c, apolipoprotein B, and apolipoprotein B/apolipoprotein A1 ratio. Second, since homocysteine level was measured 24 hours after stroke, it was unclear whether hyperhomocysteinemia is a precursor or a consequence of stroke. Furthermore, in case control studies, cases are limited to survivors of the targeted disease, which might confound the real role of candidate modifiers (Li et al. 2003). A third limitation is that the patient and control groups were statistically significantly different with respect to age, gender, and presence of HT and DM. Hence, in statistical analysis, their effects were eliminated by covariance analysis.

The following can be considered the strengths of this study: 1) results were confirmed using statistical analysis under different conditions; 2) serum vitamin levels were measured in all cases, successfully showing that vitamin levels did not differ between patients and controls; 3) the established exclusion criteria were applied in all patients and controls in order to not confound statistical analysis by variable homocysteine levels; and 4) controls were healthy patients with normal CMR.

In conclusion, hyperhomocysteinemia was more common in patients with LvAS and CES and it was directly correlated with Lp(a) in patients with LvAS. However, it was found to be a risk factor only in patients with CES in our population.

References


