Relationship between Plasma Homocysteine Levels and Saphenous Vein Graft Disease after Coronary Artery Bypass Grafts

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SUMMARY

The long-term efficacy of coronary artery bypass graft (CABG) surgery is limited by saphenous vein graft (SVG) disease. Elevated levels of plasma homocysteine are a known independent risk factor for cardiovascular disease. However, its influence on the patency of SVG is unknown. To determine whether plasma homocysteine levels are related to SVG disease after CABG we measured homocysteine levels in 80 patients who underwent CABG (age: 64±8, interval after bypass surgery: 6.4±3.1, range: 1-13 years). The patients were divided into a vein graft disease group (more than 50% angiographical stenosis in any vein graft, n=40) and a no-vein graft disease group (<50% stenosis in any vein graft, n=40). The presence of a mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene was also determined by polymerase chain reaction. Homocysteine levels in the vein graft disease group were significantly higher than in the no-vein graft disease group (11.2 vs. 9.1 µmol/l, p=0.01). Multiple regression analysis showed that the interval after CABG was an independent factor for SVG disease (odds ratio: 1.014, 95% confidence intervals: 1.003-1.025, p=0.013) and elevated levels of homocysteine tended to be an independent factor for SVG disease (odds ratio: 1.098, 95% confidence intervals: 0.994-1.213, p=0.067). There was no significant difference in MTHFR genotypes between the two groups. These findings indicate that elevated levels of plasma homocysteine are related to SVG disease after CABG. (Jpn Heart J 2001; 42: 553-562)

Key words: Saphenous vein graft disease, Homocysteine, Coronary artery bypass graft surgery

CORONARY artery bypass graft (CABG) surgery is effective in relieving the symptoms of angina and prolongs survival. However, atherosclerotic change in saphenous vein grafts (SVG) after CABG is emerging as the major determinant of long term vein graft viability.1,2) Several factors including smoking, high cholesterol levels, and diabetes mellitus have been identified as risk factors for SVG...
disease.3-5) Recently, elevated levels of homocysteine have been shown to be an independent risk factor for cardiovascular disease.6-16) Patients with homocystinuria, causing extremely high levels of homocysteine in the blood and urine due to homozygosity for cystathionine synthase deficiency, develop premature vascular disease in early adolescence.17-19) Furthermore, recent studies have demonstrated associations between plasma homocysteine levels and coronary artery disease, stroke, and peripheral vascular disease. In contrast, there have been few studies which investigated the relationship between plasma homocysteine levels and SVG disease. The purpose of this study was to determine whether plasma homocysteine levels are related to SVG disease after CABG.

**METHODS**

**Study population:** This study was conducted at the Department of Cardiology of Juntendo University Hospital in Tokyo, Japan. The study population comprised 80 patients (68 men and 12 women) who underwent elective coronary angiography more than 1 year after coronary artery bypass surgery. Clinical indications for coronary angiography included 56 patients (70%) with a new episode of angina pectoris, 4 patients (5%) with myocardial infarction, and 20 patients (25%) for other reasons (eg, cardiac evaluation before noncardiac surgery, peripheral vascular disease). Before angiography a complete medical history, including coronary risk factors, was obtained for each patient. Patients receiving medications known to affect plasma homocysteine levels (eg, multivitamins including vitamin B6, B12 and folic acid, methotrexate, anticonvulsant agents, bile acid sequestrants) were excluded from this study. The body-mass index (the weight in kilograms divided by the square of the height in meters) was calculated. Patients who had smoked within 1 year of angiography were considered current smokers. Hypertension was defined as a systolic/diastolic blood pressure of 160/95 mmHg on one or more occasions or if the patient was taking antihypertensive medications. Diabetes mellitus was diagnosed by a fasting serum glucose level of >7.0 µmol/l or an arbitrary serum glucose level of >11.1 µmol/l or if the patient was treated with insulin or oral hypoglycemic agents. Patients were excluded if they had a history of chronic renal failure or a serum creatinine level of 132.6 µmol/l or more because of a positive correlation between homocysteine and serum creatinine levels.20) Informed consent was obtained from every patient after a full explanation of the study and the Ethical Committee of the Cardiovascular Division of Juntendo University had approved the study.

**Laboratory assays:** Before angiography, blood samples were drawn after overnight fasting in tubes containing 2Na-EDTA at the time of coronary angiography. These plasma samples were stored at -80°C until they were analyzed. Total (free
plus protein bound) homocysteine levels in plasma were measured by high-performance liquid chromatography with fluorescence detection as previously described.\textsuperscript{21} Plasma levels of folate and cobalamin were measured with a chemiluminescence analyzer ACS-180.\textsuperscript{22} Pyridoxal-5’-phosphate was determined by high-performance liquid chromatography.\textsuperscript{23} Serum total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels were measured by standard enzymatic methods. Apolipoproteins (apolipoprotein A-I, B, E) were measured by immunodiffusion assay. Lipoprotein (a) was also measured according to the latex immunnoassay method.\textsuperscript{24} The crude values of low-density lipoprotein cholesterol levels were calculated using Friedewald’s formula for patients with serum triglyceride levels below 10.34 µmol/l.\textsuperscript{25}

**Genetic analysis:** Blood samples were collected in tubes containing 2Na-EDTA and DNA was extracted by a commercially available kit (DNA Extractor WB Kit). The presence of a mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene was determined by polymerase chain reaction of genomic DNA, followed by \textit{Hin}f\textsubscript{I} restriction digestion, as previously described.\textsuperscript{26} The amplified fragments were cut with \textit{Hin}f\textsubscript{I}, which can recognize the C → T substitution in the fragments. The primer generates a 198 bp fragment. If the mutation is present, \textit{Hin}f\textsubscript{I} digests the 198 bp fragment into 175 bp and 23 bp fragments. The fragments were analyzed by polyacrylamide gel electrophoresis. The wild-type allele was designated “C” and the mutant allele “T”.

**Angiographic analysis:** Selective coronary angiography was performed by a standard technique and all grafts were selectively opacified with a 5Fr catheter. Angiograms were obtained after injection of 2 mg of isosorbide dinitrate through the catheter. Images were recorded in multiple projections on 35 mm cine film. Two experienced cardiologists who were unaware of the patients' risk-factor profiles and the levels of homocysteine assessed the angiograms. Vein graft disease was defined as a stenosis of more than 50 percent of the vessel diameter in any saphenous vein graft and the rest of the patients were classified as no-graft disease. Stenoses of native coronary arteries were also categorized according to the reporting system proposed by the American Heart Association.\textsuperscript{27} Lesions of anastomosis between SVG and a native artery were excluded from this study.

**Statistical analysis:** All data are expressed as the mean±SD except for homocysteine and lipoprotein (a) levels, which are presented as the median and range. Because the distributions of values for homocysteine and Lp (a) were skewed, these data were analyzed by a non-parametric statistical procedure (Mann-Whitney method). Other data were analyzed using unpaired Student \textit{t} tests for continuous variables, and the chi-square statistic test for categorical variables to compare between groups. The odds ratio and 95% confidence intervals were estimated with a stepwise logistic regression model, in which age, interval between
bypass surgery and coronary angiography, total cholesterol, high density lipoprotein cholesterol, lipoprotein (a) and homocysteine were included as confounding variables. A value of \( p < 0.05 \) was considered statistically significant. Statistical analyses were performed with commercially available statistic software (Stat View version 5.0 ABACUS concepts, Inc. SPSS version 4.0 for Macintosh).

**RESULTS**

The clinical characteristics of the patients are summarized in Table I. In all patients, the mean interval between bypass surgery and coronary angiography was 6.4 years (SD:±3.1 years, range: 1-13 years). There were no significant differences in mean age, conventional risk factors, the number of diseased native coronary vessels, and interval between bypass surgery and coronary angiography, and percentage of myocardial infarction before CABG between the vein graft disease group and the no-graft disease group.

Figure 1 shows the frequency distribution of plasma homocysteine levels (\( \mu \text{mol/l} \)) in all patients. The distribution of plasma homocysteine levels was mildly skewed to the lower level. Median homocysteine levels were 10.1 \( \mu \text{mol/l} \) (range: 3.8-30.7 \( \mu \text{mol/l} \)).

Figure 2 shows the plasma homocysteine levels in patients in the vein graft disease group and the no-graft disease group. Plasma homocysteine levels in the vein graft disease group were significantly higher than in the no-graft disease group (median levels: 11.2 vs 9.1 \( \mu \text{mol/l} \), \( p = 0.01 \)).

**Table I. Clinical Characteristics of Patients with or without Saphenous Vein Graft Disease**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Graft disease group (n=40)</th>
<th>No-graft disease group (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64±8</td>
<td>65±8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.5±3.1</td>
<td>24.5±2.8</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>53</td>
<td>42</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>45</td>
<td>52</td>
</tr>
<tr>
<td>Numbers of diseased vessels (%)</td>
<td>2.6</td>
<td>2.6</td>
</tr>
<tr>
<td>Period after CABG (years)</td>
<td>6.5±2.9</td>
<td>5.6±3.2</td>
</tr>
<tr>
<td>Myocardial infarction (%)</td>
<td>60</td>
<td>58</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SD and percentage. CABG=coronary artery bypass grafting.
The levels of total cholesterol, triglyceride, and low-density lipoprotein cholesterol were not different between the two groups (Table II). However, high density lipoprotein cholesterol levels were significantly lower in the vein graft disease group than in the no-graft disease group (0.93±0.28 vs. 1.19±0.36 µmol/l, p<0.01).

The MTHFR genotype was analyzed by polymerase chain reaction in 52 patients (Table III). The genotype prevalence of both groups was compatible with Hardy-Weinberg equilibrium. In all patients, the frequencies of the three genotypes were C/C; 35%, C/T; 52%, and T/T; 13%, resulting in a similar prevalence in both groups. We found no significant association between SVG disease and MTHFR genotype.

Logistic regression analysis showed that the interval between bypass surgery and coronary angiography independently correlated with SVG disease (odds ratio: 1.014, 95% confidence intervals: 1.003-1.025, p=0.013) and elevated levels of homocysteine tended to be an independent factor for SVG disease (odds ratio: 1.098, 95% confidence intervals: 0.994-1.213, p=0.067) (Table IV).
Table II. Comparison of Lipid and Glycemic Measurements in Patients with or without Saphenous Vein Graft Disease

<table>
<thead>
<tr>
<th></th>
<th>Graft disease group</th>
<th>No-graft disease group</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cho (µmol/l)</td>
<td>5.02±0.96</td>
<td>4.86±0.75</td>
</tr>
<tr>
<td>TG (µmol/l)</td>
<td>1.71±1.09</td>
<td>1.62±1.20</td>
</tr>
<tr>
<td>HDL-c (µmol/l)</td>
<td>0.98±0.26 *</td>
<td>1.16±0.31</td>
</tr>
<tr>
<td>LDL-c (µmol/l)</td>
<td>3.10±0.85</td>
<td>2.84±0.67</td>
</tr>
<tr>
<td>apo A-I (µmol/l)</td>
<td>106±23</td>
<td>114±24</td>
</tr>
<tr>
<td>apo B (µmol/l)</td>
<td>107±31</td>
<td>107±21</td>
</tr>
<tr>
<td>apo E (µmol/l)</td>
<td>6.1±1.6</td>
<td>5.8±2.8</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)(median)</td>
<td>23.0</td>
<td>17.8</td>
</tr>
<tr>
<td>FBS (µmol/l)</td>
<td>6.44±1.66</td>
<td>5.94±1.83</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.8±1.1</td>
<td>6.5±1.7</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SD, median and percentage. T-cho=total cholesterol; TG=triglyceride; HDL-c=high-density lipoprotein cholesterol; LDL-c=low-density lipoprotein cholesterol; Lp(a)=lipoprotein (a); FBS=fasting blood sugar, *p=0.01.

Figure 2. Median plasma homocysteine levels in patients in the vein graft disease group and in the no-vein graft disease group. Plasma homocysteine levels in patients in the vein graft disease group were significantly higher than in the no-vein graft disease group (11.2: 9.1 µmol/l; p=0.01).
DISCUSSION

CABG has become widely accepted and established as an effective therapy for coronary artery disease. However, its long-term efficacy is limited by SVG disease.1,2) Many risk factors responsible for SVG disease, including smoking, elevated levels of cholesterol, diabetes and longer intervals after CABG, have been identified.3-5) In this study, we have demonstrated for the first time that homocysteine levels were significantly higher in the vein graft disease group than in the no-vein graft disease group, indicating that elevated levels of plasma homocysteine are an independent risk for SVG disease.

Most case-control and prospective studies have demonstrated a positive association of plasma homocysteine levels with atherosclerotic vascular diseases and thrombotic diseases.6-16) In the recent British United Provident Association prospective study, the risk of coronary artery disease among men in the highest quartile of serum homocysteine was 2.9 times higher than men in the lowest quartile.16) A meta-analysis has suggested that a 5 µmol/l increment in the plasma

Table II. Levels of Plasma Homocysteine and Vitamins in Patients with or without Saphenous Vein Graft Disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>Graft disease group</th>
<th>No-graft disease group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>11.2 (3.8-30.7) *</td>
<td>9.1 (5.4-21.0)</td>
</tr>
<tr>
<td>Folate (ng/ml)</td>
<td>8.3±3.0</td>
<td>7.1±2.8</td>
</tr>
<tr>
<td>Vitamin B12 (pg/ml)</td>
<td>750.5±373.0</td>
<td>764.5±353.0</td>
</tr>
<tr>
<td>Vitamin B6 (ng/ml) median</td>
<td>10.3</td>
<td>8.8</td>
</tr>
<tr>
<td>MTHFR genotype (%)</td>
<td>30/53/17</td>
<td>36/56/8</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SD, median and percentage. MTHFR=methylene-tetrahydrofolate reductase, *p = 0.01.

Table IV. Stepwise Logistic Regression Analysis in 80 Patients, with SVG Disease as the Dependent Variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period after CABG</td>
<td>1.01*</td>
<td>1.003-1.025</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>1.098**</td>
<td>0.994-1.213</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.96***</td>
<td>0.92-1.01</td>
</tr>
</tbody>
</table>

Variables: homocysteine, HDL-cholesterol (high-density lipoprotein cholesterol), age, period after CABG (coronary artery bypass grafting), total cholesterol, lipoprotein (a) CI=confidence intervals, *p=0.013, **p=0.07, ***p=0.15.
homocysteine level is associated with a 60% higher prevalence of coronary artery disease.\(^8\) Thus plasma homocysteine levels have been considered to be a strong predictor of coronary artery disease in previous studies, however, it has not been proven whether a high plasma homocysteine level is a predictor of the presence of SVG disease after CABG. In this study population, plasma homocysteine levels in all subjects were relatively high because of the presence of coronary artery disease. This shift in plasma homocysteine levels may be the reason the difference between the two groups diminished.

It is well known that SVGs may result from not only atherosclerosis but also thrombosis and diffuse intimal hyperplasia.\(^{28,29}\) Although the exact mechanisms of homocysteine leading to atherosclerosis have not been fully elucidated, experimental studies have demonstrated that high homocysteine levels lead to endothelial dysfunction and thrombus formation.\(^{30-35}\) These mechanisms of homocysteine may be related to SVG disease after CABG.

Eritsland, et al\(^{10}\) previously investigated the association between homocysteine levels and graft patency during the short term (within 1 year) after CABG and reported no relation between homocysteine levels and the prevalence of total occlusion in bypass grafts. In our study, the analysis of the association between homocysteine levels and angiographical stenotic lesion \(\geq 50\%\) in SVG during a longer term after CABG may reveal results different to theirs.

Several reports have emphasized the relationship between SVG disease and conventional coronary risk factors, and intervention trials have also provided strong evidence for aggressive treatment of dyslipidemias with pharmacological agents as the most efficient therapeutic approach.\(^{3,5,36,37}\) In the present study, in addition to high levels of homocysteine, low levels of high-density lipoprotein cholesterol and longer intervals between bypass surgery and coronary angiography were considered to be risk factors for SVG disease. In contrast, low-density lipoprotein cholesterol has no significant relation with SVG disease in this study because levels of low-density lipoprotein cholesterol were controlled by lipid lowering drugs in 64% of all subjects.

The present study has several limitations. The study is a retrospective and observational analysis of patients who were referred for coronary angiography, and there was a small number of patients.

**CONCLUSION**

Although the small sample size prevents us from drawing a definite conclusion, the results of the present study suggest that an elevation of homocysteine levels is related to SVG disease after CABG. Further investigations are warranted to confirm the atherogenic role of homocysteine in SVG disease in a large
sample size, and studies are needed on whether homocysteine lowering therapy, such as folate supplementation, results in deceleration of the progression of SVG disease.

REFERENCES