Impact of Elevated Plasma Total Homocysteine Concentration on Coronary Atherosclerosis in Chinese Patients With Acute Myocardial Infarction Undergoing Primary Coronary Intervention

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SUMMARY

Elevated plasma total homocysteine (tHcy) has been considered to be a new risk factor for coronary atherosclerotic disease. However, the association has not been proven indisputably, and the strength of the relationship and the interaction of plasma tHcy with other conventional risk factors remain uncertain in the clinical setting of acute myocardial infarction (AMI). The aim of this study was to investigate whether an elevated plasma level of tHcy is an independent predictor of the late stage of coronary atherosclerotic lesions in Chinese patients with AMI, who are undergoing primary percutaneous coronary intervention (PCI).

Plasma levels of tHcy were prospectively measured in 178 consecutive patients with ST-segment elevated AMI undergoing primary PCI. The plasma level of tHcy was also measured in 30 control subjects with normal coronary angiographic findings. The plasma level of tHcy was significantly higher in the patients with AMI than in control subjects (10.5 ± 3.3 µmol/L versus 8.3 ± 2.4 µmol/L, P = 0.0004). Multiple stepwise logistic regression analysis of the baseline characteristics demonstrated that smoking (P = 0.004) and creatinine level (P < 0.0001) were independent predictors of an elevated plasma level of tHcy. Moreover, an increased plasma level of tHcy (P = 0.003), female gender (P = 0.008), diabetes mellitus (P = 0.020), and the presence of previous myocardial infarction (P = 0.003) were independent predictors of the late stage of multivessel diffuse atherosclerosis (defined by ≥ 2 epicardial vessels with moderate or severe diffuse atherosclerosis). In conclusion, this investigation supports the hypothesis that a raised homocysteine concentration is a strong risk factor for the late stage of diffuse coronary atherosclerosis.
in Chinese patients with AMI undergoing primary PCI. This result therefore raises the prospect of the need for major therapeutic research in Chinese patients. (Int Heart J 2005; 46: 181-193)

**Key words:** Hyperhomocysteinemia, Diffuse coronary atherosclerosis, Acute myocardial infarction

ELEVATED plasma total homocysteine (tHcy) has been suggested to represent a new important risk factor for atherosclerotic vascular disease.\(^1\)\(^2\) Even mild to moderate hyperhomocysteinemia (hHcy) (13 to 25 \(\mu\)mol/L),\(^3\)\(^4\) regardless of the underlying etiology, has been shown to be strongly associated with obstructive cardiovascular disease,\(^5\)\(^6\) with restenosis and major adverse cardiac events after coronary angioplasty,\(^7\) and with all-cause mortality.\(^8\)\(^9\) However, despite the consistency of various case-control and prospective studies\(^5\)\(^6\)\(^8\)\(^9\) involving thousands of subjects, methodological problems make difficult a certain determination of the strength of these relationships, and in particular of the independence of, or interactions between, elevated plasma tHcy and traditional risk factors.\(^10\)

Accordingly, the absolute risk of tHcy for atherosclerotic coronary lesions remains undetermined, especially for patients with acute myocardial infarction (AMI) undergoing primary percutaneous coronary intervention (primary PCI).

Plasma tHcy levels are determined by both genetic and nutritional factors.\(^11\)

Fresh vegetables and fruit are major dietary sources of the antioxidants, carotenoids, vitamin C and folate, and vegetable oils are the main source of vitamin E. A low folate status is associated with elevated plasma tHcy concentration.\(^12\)

Cultural differences exist between western and eastern countries in the consumption of meat, fruit, and vegetables. The incidence of hHcy is estimated to be 13% to 16% in the general population in the United States.\(^13\) However, no data are available on the incidence of hHcy in the Chinese population. Additionally, the relationship between elevated plasma tHcy and severity of coronary atherosclerosis in Chinese patients has not been reported. Therefore, elucidation of these issues is of essential importance, especially in patients with AMI who are undergoing primary PCI. Understanding these issues will promote an understanding of the effect of elevated plasma hHcy on atherosclerotic-occlusive coronary disease and information about when, and above which plasma tHcy level patients should be considered for treatment by folic acid. Hence, this investigation aimed to elucidate the effect of elevated plasma tHcy concentration on coronary atherosclerotic lesions and the incidence of elevated plasma tHcy concentration in Chinese patients with AMI undergoing primary PCI.
METHODS

Study population and inclusion criteria: In our hospital, all patients with AMI are eligible for primary PCI. For the purposes of this study, the plasma tHcy level of all patients who underwent primary PCI was prospectively measured. A blood sample was drawn following vascular puncture, before coronary angiography was performed in the cardiac catheterization laboratory.

To avoid other variables that could influence the plasma concentration of tHcy, we excluded patients with a history of folic acid or vitamin B complex supply, history of folic acid or vitamin B complex deficiency, and renal insufficiency (creatinine $\geq 1.5 \text{ mg/dL}$), as well as fasting less than 4.5 hours before blood sampling. Between November 2002 and December 2003, we prospectively investigated and recruited 199 consecutive patients of various ages who presented with AMI of $< 12$ hours duration undergoing primary PCI in our hospital. Twenty-one (10.6%) of the 199 patients were subsequently excluded because they fasted for less than 4.5 hours (11 patients); had a history of current vitamin B complex utilization (3 patients), or exhibited renal function impairment (7 patients). Therefore, the remaining 178 patients constituted the study population. Informed consent was obtained from all study subjects. Thirty subjects who underwent coronary angiography due to angina with normal coronary angiographic findings served as the control subjects. The study protocol was approved by the Institutional Review Committee on Human Research of our hospital.

Blood sampling and laboratory investigations: One blood sample was obtained from each patient in the catheterization laboratory prior to coronary angiography. The tHcy was centrifuged immediately after the blood sample was drawn. It was then stored at - 4°C and measured in less than 6 hours after the blood sample was drawn by Fluorescence Polarization immunoassay (FPIA) (Abbott IMx, Abbott Diagnostics). The lower detection limit of this test was 0.00 $\mu\text{mol/L}$ and a plasma tHcy level $\geq 13 \mu\text{mol/L}$ was considered as hHcy in our hospital. The intraindividual variability of plasma tHcy levels in patients and angina subjects was assessed. The mean intra-assay coefficients of variance were 3.83% and 3.24%, respectively.

Angiographic analysis and definitions: Quantitative angiographic analysis of the percentage of minimal lumen diameter stenosis, the lesion length, and the reference lumen diameter were conducted using a digital edge-detection algorithm (DUQUE System) and by selecting end-diastolic frames, to demonstrate the stenosis in its most severe and non-foreshortened projection. The contrast-filled guiding catheter was used as the calibration standard, and the reference and minimal lumen diameters were calculated before and after angioplasty.

AMI was defined as typical chest pain that lasted for more than 30 minutes with ST-segment elevation $> 1 \text{ mm}$ in two consecutive precordial or inferior
leads. Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in meters (kg/m²).

Multivessel disease was defined by stenoses of > 50% in ≥ 2 major epicardial coronary arteries. A plaque lesion was defined as any atherosclerotic plaque obstruction of ≥ 35% but < 50% in one or more major epicardial coronary arteries. Multivessel plaque lesions were defined by ≥ 2 atherosclerotic plaque obstructions of ≥ 35% but < 50% in ≥ 2 epicardial coronary arteries.

Classification of severity of coronary atherosclerosis according to angiographic morphologic features of coronary artery trees: Single-vessel diffuse atherosclerosis was defined as ≥ 50% of the length of one epicardial vessel with atherosclerotic change. Multivessel diffuse atherosclerosis was defined as ≥ 50% of the length of ≥ 2 epicardial vessels with atherosclerosis.

A “scoring system” from 0 to 3 was prospectively designed to recognize angiographic morphologic features of coronary artery atherosclerosis, and thus determine its severity. A score of 0 referred to a normal angiographic appearance or a situation in which less than 50% of the length of the epicardial vessel exhib-

![Image](https://via.placeholder.com/150)

**Figure 1.** A: Left coronary angiography demonstrated mild diffuse atherosclerosis through ≥ 50% of the length of the left anterior descending artery (LAD) (black arrow heads). B: Right coronary angiography demonstrated a plaque rupture in the proximal right coronary artery (RCA) (black arrow heads) and thrombus formation was observed from the proximal to distal RCA (black arrows). C: Mild diffuse atherosclerosis through ≥ 50% of the length of the RCA was observed following primary percutaneous coronary intervention (PCI) (black arrow heads). These angiographic morphologic features indicated a score of 1 of diffuse atherosclerosis. D: Left coronary angiography demonstrated a normal appearance of the LAD and left circumflex artery. E: Right coronary angiography demonstrated a plaque rupture in the mid RCA with thrombus occlusion of the mid RCA and proximal posterolateral artery (black arrow heads). F: Normal appearance of RCA was observed following primary PCI. These angiographic morphologic features indicated a score of 0 of coronary atherosclerosis.
itated atherosclerosis (Figure 1). A score of 1 was defined as mild diffuse atherosclerosis through $\geq 50\%$ of the length of the epicardial vessel (such that the angiographic morphologic feature of the coronary artery was only mildly irregular) (Figure 1). A score of 2 was defined as moderate diffuse atherosclerosis through $\geq 50\%$ of the length of the epicardial vessel (and the angiographic morphologic feature indicated that the diffuse atherosclerotic area had a relative smaller luminal diameter than the normal reference luminal diameter) (Figure 2). A score of 3 was defined as severe diffuse atherosclerosis through $\geq 50\%$ of the length of the epicardial vessel (and the angiographic morphologic features showed that the atherosclerotic vessel had a diffuse tubular obstruction and that the normal reference luminal diameter was lost) (Figure 2).

Late stage of multivessel diffuse atherosclerosis was defined by $\geq 2$ epicardial vessels with a diffuse atherosclerosis score of $\geq 2$.

**Figure 2.**

A: Right coronary angiography demonstrated a plaque rupture near the ostium of the right coronary artery (RCA) with intracoronary thrombus formation (black arrows). Moderate diffuse atherosclerosis through $\geq 50\%$ of the length of the RCA was observed (black arrow heads). B: Left coronary angiography demonstrated moderate diffuse atherosclerosis through $\geq 50\%$ of the length of the left anterior descending artery (LAD) (small black arrows), left circumflex artery (black arrow heads), and first diagonal branch (bigger black arrows). These angiographic findings indicated a score of 2 of diffuse coronary atherosclerosis. C: Right coronary angiography demonstrated severe diffuse atherosclerosis of the RCA (black arrows). A plaque rupture in the distal RCA was noted with intracoronary thrombus formation (black arrow heads). D: Left coronary angiography demonstrated severe diffuse atherosclerosis of the LAD (black arrow heads) and a plaque rupture was observed in the proximal LAD (black arrows). These angiographic findings indicated a score of 3 of diffuse atherosclerosis.
Data collection: Detailed in-hospital and follow-up data including age, sex, coronary risk factors, Killip score on admission, BMI, creatinine level, plasma thcy level, and number of diseased vessels were obtained. Angiographic morphologic features indicated plaque lesions and the severity of coronary artery diffuse atherosclerosis. These data were collected prospectively and entered into a computerized database.

Statistical analysis: Data are expressed as the mean ± SD. Categorical variables were compared using the Chi-square test or Fischer’s exact test. Univariate analyses were performed using Student’s t test. Continuous variables among 3 groups were compared using one-way ANOVA. Scheffe’s test was used for nonparametric multiple comparison. Multiple linear regression analysis was used for evaluation of variables in predicting an increased plasma thcy level. Statistical analysis was performed using SAS statistical software for Windows version 8.2 (SAS Institute, Cary, NC). A probability value < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics and angiographic results of study patients and control subjects (Table I): Table I depicts the baseline characteristics of both study patients and normal control subjects. The study patients were older than the control subjects. A significantly larger proportion of study patients than control subjects were male. Except for hypercholesterolemia, other traditional coronary risk factors, including hypertension, diabetes mellitus, and current smoking were more prevalent in study patients than in control subjects. Less than 10.0% of the patients had previously suffered from a stroke or previous MI. The BMI of the study patients markedly exceeded that of the control subjects. Moreover, a laboratory investigation demonstrated that the plasma thcy and creatinine levels of the study patients were substantially higher than those of the control subjects.

Angiographic findings demonstrated that control subjects had normal coronary arteries. However, over 52% of the instances of anterior wall myocardial infarction and nearly 50% of the instances of multivessel disease were found in the study patients. Additionally, the incidences of multivessel plaque lesions and severe multivessel diffuse atherosclerosis were found to be 50.0% and 79.8%, respectively.

Cardiogenic shock was observed in 11.2% (20) of the patients upon presentation. The 30-day mortality was 11.2%.

Multiple stepwise logistic regression analysis of enrolled baseline variables in Table I demonstrated that age [odds ratio (OR): 1.05, 95% confidence interval (CI): 1.01 - 1.10, \( P = 0.016 \)], BMI (OR: 1.75, 95% CI: 1.38 - 2.17, \( P < 0.0001 \)),...
and creatinine level (OR: 14.3, 95% CI: 4.2 - 100, \( P = 0.0008 \)) were independent predictors of an elevated plasma tHcy level.

**Baseline characteristics and laboratory findings of study patients divided into three categories of homocysteine levels (Table II):** The study patients were divided into three groups according to the plasma tHcy levels in Table II. The hHcy (\( \geq 13 \) \( \mu \text{mol/L} \)) was found to present in 23.0% of our patients. There were no significant differences in terms of age, hypertension, diabetes mellitus, hypercholesterolemia, previous stroke, previous myocardial infarction, BMI, anterior wall infarction, or 30-day mortality among these 3 groups of patients. However, a significantly lower incidence of male gender was noted in the lowest tHcy group of patients. Furthermore, the incidence of current smoking was substantially higher in the middle tHcy group and in the highest tHcy group of patients than in the lowest tHcy group of patients. Moreover, the creatinine level was markedly elevated in the highest tHcy group of patients than in the other two groups of the patients.
Multiple linear regression analysis of relevant baseline variables for predicting elevated plasma total homocysteine level (Table III): The independent predictors of elevated plasma tHcy level in the study patients are shown in Table III. Multiple linear regression analysis of enrolled baseline variables demonstrated that only current smoking and creatinine level were significant independent predictors of an elevated plasma tHcy level.

Correlations between baseline variables, laboratory findings of study patients and extent of coronary atherosclerotic lesions (Tables IV and V): As shown in Table IV, univariate analysis showed only a weak association between the baseline characteristics, laboratory findings, and multivessel disease. Additionally, the analysis demonstrated only a statistically significant difference between the diabetes mellitus, previous myocardial infarction, and multivessel plaque lesions. Furthermore, when a plasma tHcy level of ≥ 13 µmol/L was taken as hHcy, the correlation between multivessel disease (P = 0.193), multiplaque lesions (P = 0.060), and hHcy did not reach statistical significance. However, univariate analysis demonstrated that the enrollment variables strongly related to the late stage of multivessel diffuse atherosclerosis were age, male gender, diabetes mellitus, previous stroke, previous MI, and an elevated plasma tHcy level.
Using multiple stepwise logistic regression analysis (Table V), only an elevated plasma tHcy level was strongly associated with multivessel plaque lesions. Furthermore, the only independent variables that were related to the late stage of multivessel diffuse atherosclerosis were female gender, diabetes mellitus, previous MI, and an elevated plasma tHcy level.
DISCUSSION

Plasma tHcy levels are well recognized to be determined by both genetic and nutritional factors, as well as dietary habits.\textsuperscript{11)} An elevated plasma tHcy level can follow chronic alcoholic consumption or impaired liver function, resulting in altered methionine and homocysteine metabolism.\textsuperscript{16)} Additionally, plasma tHcy levels are strongly related to sex, age, smoking, serum folate, vitamin B12, and renal function.\textsuperscript{17-19)} One of the important findings in this study was that the mean plasma tHcy level was significantly higher in patients with AMI than in control subjects with normal coronary arteries. Furthermore, the results of this study have demonstrated that age, BMI, current smoking, and creatinine level were independently related to the plasma tHcy level. These findings agree with those of previous studies,\textsuperscript{17-19)} and further confirm that hyperhomocysteinemia is clearly multifactorial.\textsuperscript{16-19)}

The level at which plasma tHcy is considered raised and deleterious remains uncertain. However, it has been taken to be around 13 $\mu$mol/L for mild hHcy.\textsuperscript{17,20)} The mean plasma tHcy concentration in our patients was below this level and only 15.7% of our patients had a plasma tHcy of above 13 $\mu$mol/L. Our findings indicated that although cultural differences and differences in consumption of food exist between western and eastern countries, the Chinese patient population exhibited a similar prevalence of hHcy to that of the Western patient population.\textsuperscript{13,17)} This fact may explain, in part, why age, current smoking, and

Table V. Multiple Stepwise Logistic Regression Analysis of Baseline Characteristics and Laboratory Findings in Predicting Multivessel Disease, Multivessel Plaque Lesions, or Severe Multivessel Diffuse Atherosclerosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Multivessel disease OR (95% CI)</th>
<th>Multivessel plaque lesions OR (95% CI)</th>
<th>Severe diffuse atherosclerosis* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>1.14 (0.36 - 2.14)</td>
<td>1.82 (0.21 - 1.39)</td>
<td>2.22 (1.09 - 4.55)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.45 (0.78 - 2.70)</td>
<td>1.56 (0.82 - 2.97)</td>
<td>2.01 (1.12 - 3.62)</td>
</tr>
<tr>
<td>Previous MI</td>
<td>2.65 (0.75 - 9.39)</td>
<td>2.94 (0.74 - 11.69)</td>
<td>4.16 (1.39 - 12.48)</td>
</tr>
<tr>
<td>Plasma tHcy level†</td>
<td>1.02 (0.93 - 1.12)</td>
<td>1.11 (1.00 - 1.23)</td>
<td>1.15 (1.05 - 1.25)</td>
</tr>
</tbody>
</table>

*Indicates severe multivessel diffuse atherosclerosis; † continuous value of plasma tHcy level was used as the variable during multivariable analysis.

CI = confidence interval; LDL = low-density lipoprotein cholesterol; MI = myocardial infarction; OR = odds ratio; tHcy = plasma total homocysteine.
renal function were independently related to elevated plasma tHcy levels in this and other studies,\textsuperscript{17-19} when the supply of folate and vitamin B12 was sufficient.

Elevated plasma tHcy is considered to be an independent risk factor for atherosclerotic vascular disease.\textsuperscript{1,2,5,6} However, the association has not indisputably been proven,\textsuperscript{21} and the mechanisms by which Hcy may be atherogenic have only been elucidated based on biological mechanisms in \textit{in vitro} studies.\textsuperscript{22-24} On the other hand, the human evidence mainly comes from case-control studies\textsuperscript{10,25,26} whereas population-based prospective cohort studies\textsuperscript{6,27} have given conflicting results. Furthermore, data is still lacking regarding the impact of plasma tHcy on coronary atherosclerotic lesions in the clinical setting of AMI under primary PCI.

Another important finding in this study was that when the continuity of plasma tHcy concentration was used as the variable in univariable analysis, it was not a significant predictor of multivessel disease and was only weakly associated with multivessel plaque lesions. Additionally, when a plasma tHcy level of > 13 \(\mu\text{mol/L}\) was taken as hHcy, the correlation between multivessel disease, multi-plaque lesions, and hHcy did not reach statistical significance. This fact may partially explain why previous reports on the absolute risk of tHcy to atherosclerotic cardiovascular disease have presented conflicting results.\textsuperscript{1,2,5,6,10,27,28} In the present study, the most important finding was that the plasma tHcy level was strongly associated with the late stage of multivessel diffuse atherosclerosis, suggesting that a raised plasma tHcy level may influence the function and integrity of endothelial cells with a widespread pattern throughout the coronary tree, and finally presenting with the typically angiographic morphologic feature of diffuse coronary atherosclerosis. This suggestion is based on the findings of this study which demonstrated that the plasma tHcy level was significantly higher in study patients who had severe multivessel diffuse disease than in normal control subjects who had normal coronary arteries. This suggestion is further supported by growing evidence that Hcy is toxic to the endothelium, increases the risk of thrombosis, increases the production of collagen, and decreases the availability of nitric oxide.\textsuperscript{22-24}

In this investigation, multiple stepwise logistic regression analysis demonstrated that female gender, diabetes mellitus, and previous MI were the other independent predictors of the late stage of multivessel diffuse atherosclerosis. These findings reveal that plasma tHcy level, female gender, diabetes mellitus, and previous MI predict the more extended and advanced stage of coronary atherosclerosis.

The causal role of Hcy in atherosclerotic disease remains to be identified. Previous researchers have claimed that atherosclerosis is a lipid-driven disease and that hHcy cannot in itself initiate the process.\textsuperscript{29} Creatinine levels are well known to increase when the atherosclerotic process impairs renal function. This
Investigation revealed a strong effect of creatinine on the plasma tHcy level. Moreover, plasma tHcy levels were strongly related to the accumulation of coronary atherosclerosis. Accordingly, this investigation supports the idea\textsuperscript{29} that elevated plasma tHcy concentration is an aftermath, rather than a cause, of coronary atherosclerotic disease.

There are several limitations to this study. First, the inferences made concerning the stage of coronary atherosclerosis in this study are based on the angiographic morphologic findings but are not based on intravascular ultrasound studies. The sensitivity of intravascular ultrasound may reinforce the claim of an association between plasma tHcy level and the severity of coronary atherosclerosis. Hence, the sensitivity herein of the diagnosis of the stage of coronary atherosclerosis may not be optimal. Second, without the support of cellular and molecular biologic studies, any suggestion that increased plasma tHcy concentration is an effect rather than a cause of coronary atherosclerotic disease is speculative. Finally, the selection of a plasma tHcy level of $> 13 \, \mu\text{mol/L}$ indicating hHcy in this study was arbitrary. However, previous studies took this value as hHcy.\textsuperscript{17,20}

In conclusion, an increased plasma tHcy level represents an independent risk of the accumulation of coronary atherosclerosis similar to previous MI, female gender, and diabetes mellitus. Prospective studies are required to evaluate the efficacy of plasma tHcy-lowering therapy in preventing the late stage of coronary atherosclerosis in the Chinese patient population.

\textbf{REFERENCES}

13. Nallamothu BK, Fendick AM, Omenn GS. Homocyst(e)ine and coronary heart disease: pharmacoeconomic support for interventions to lower hyperhomocyst(e)inemia. Pharmacoeconomics 2002; 20: 429-42. (Review)