Value and Level of Plasma Homocysteine in Patients With Angina Pectoris Undergoing Coronary Angiographic Study

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SUMMARY

This study tested whether the plasma level of total homocysteine (tHcy) was predictive of obstructive coronary artery disease (CAD) and clinical outcome in patients undergoing coronary angiographic (CAG) study. From September 2002 to October 2004, 1,305 consecutive patients with angina pectoris undergoing CAG study were consecutively enrolled. Blood samples were prospectively collected to assess the plasma level of tHcy from each patient before catheterization. Of these 1305 patients, 676 (51.8%) had multivessel disease (group 1), 367 (28.1%) had single-vessel disease (group 2), and 262 (20.1%) had normal coronary artery or insignificant coronary artery disease (group 3). The plasma level of tHcy was notably higher in group 1 than in groups 2 and 3 (11.6 ± 4.4 versus 10.9 ± 4.0 versus 10.4 ± 3.8 , $P < 0.001$). Univariate binary logistic regression analysis demonstrated that the plasma tHcy level was strongly associated with multiple-vessel disease (MVD) (defined as ≥ 2 vessel disease) ($P < 0.001$). Multivariate binary logistic regression analysis showed that tHcy level, fasting blood sugar, diabetes mellitus, and age were significantly and independently predictive of MVD (all $P < 0.03$). Univariate Cox regression analysis demonstrated that tHcy level was predictive of long-term mortality ($P = 0.042$). However, the tHcy level was not an independent predictor of long-term mortality on multivariate Cox regression analysis ($P > 0.05$). The results of our study support the hypothesis that tHcy level is an independent predictor of MVD in patients with chest pain undergoing CAG study. Conversely, our study did not support the tHcy level as an independent predictor of long-term mortality in this clinical setting. (Int Heart J 2011; 52: 280-285)

Key words: Angina pectoris, Coronary artery disease, Plasma tHcy level, Long-term clinical outcome

Despite the implementation of primary and secondary strategic management, coronary artery disease (CAD) remains the leading cause of death worldwide.^{1,2} Interestingly, while traditional CAD risk factors have been suggested to be one of the most useful predictors of future CAD risk, they can only predict less than half of future cardiovascular events and cardiovascular disease.^{3,4} Of these novel CAD risk factors, C-reactive protein (CRP) is believed to be particularly important in predicting future cardiovascular events because not only is CRP a biomarker, but it is also found to directly participate in the initiation and propagation of arterial atherosclerosis.⁵⁻¹³ In contrast, the reliability of plasma total homocysteine (tHcy) in either predicting future cardiovascular events or assessing the severity of CAD is still controversial because of inconsistent results from previous studies.¹⁴⁻¹⁶ Therefore, to test the correlation between the plasma level of tHcy and severity of CAD as well as the clinical outcome in patients with chest pain undergoing coronary angiographic study, the current study consecutively enrolled 1305 patients

who were followed-up for more than one year.

METHODS

Study population and inclusion criteria: For the purposes of this study, the plasma tHcy level of all patients who had angina pectoris undergoing cardiac catheterization was prospectively measured. A single blood sample was obtained following vascular puncture and before coronary angiography in the cardiac catheterization laboratory. To avoid other variables that could potentially influence the plasma concentration of tHcy, we excluded patients with a history of consumption of folic acid or vitamin B supplements, history of folic acid or vitamin B complex deficiency, renal insufficiency (creatinine ≥ 1.5 mg/dL), and fasting less than 4.5 hours before blood sampling. Between September 2002 and October 2004, we prospectively recruited 1,305 consecutive patients of various ages who underwent coronary angiographic study due to chest pain accord-

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ing to the criteria at our hospital. Of these 1305 patients, 676 (51.8%) had multivessel disease (group 1), 367 (28.1%) had single-vessel disease (group 2), and 262 (20.1%) had normal coronary arteries or insignificant coronary artery disease (group 3). The study protocol was approved by the Institutional Review Committee on Human Research at our institution.

Blood sampling and laboratory investigations: One blood sample was obtained from each patient in the catheterization laboratory prior to coronary angiographic examination. After immediate centrifugation of the blood sample, the plasma was stored at -4°C and then the tHcy level was determined by Fluorescence Polarization immunoassay (FPIA) (Abbott IMx, Abbott Diagnostics) within 6 hours.^{17,18)} The lower detection limit of this test was $0.00\text{ }\mu\text{mol/L}$ and a plasma tHcy level $> 12\text{ }\mu\text{mol/L}$ was considered above the normal limit in our laboratory. Assessment of intraindividual variability in plasma tHcy levels showed a mean intra-assay coefficient of variance of 3.35%.

Angiographic analysis and definitions: Quantitative angiographic analysis of the degree of coronary artery luminal stenosis and the reference lumen diameter was conducted using a digital edge-detection algorithm (DUQUE System) by selecting end-diastolic frames to demonstrate stenosis in its most severe and non-foreshortened projection.¹⁹⁾ With the contrast-filled guiding catheter serving as the calibration standard, the reference and minimal lumen diameters were determined before and after angioplasty. Single-vessel disease was defined as stenoses of $> 50\%$ in 1 major epicardial coronary artery. Multivessel disease was defined as stenoses of $> 50\%$ in ≥ 2 major epicardial coronary arteries. Body mass index (BMI) was defined as body weight in kilograms divided by the square of body height in meters (kg/m^2). A higher plasma concentration of tHcy was defined as one above the median level (ie, $10.29\text{ }\mu\text{mol/L}$), whereas a lower plasma level was defined as one equal or less than the median level (ie, $10.29\text{ }\mu\text{mol/L}$).

Data collection: Detailed in-hospital and follow-up data including age, gender, coronary risk factors, BMI, concentrations of serum creatinine and plasma tHcy, number of diseased vessels, and the incidences of percutaneous coronary intervention (PCI) and coronary artery bypass surgery (CABG) were obtained. In-hospital and long-term mortality were recorded during hospitalization and follow-up in our outpatient department. These data were collected prospectively and entered into a digital database.

Statistical analysis: Data are expressed as the mean $\pm$ SD. Differences in continuous variables were analyzed by independent *t* test and categorical variables by the chi-square test. Continuous variables among 3 groups were compared using one-way ANOVA followed by the Bonferroni multiple comparison procedure. The baseline variables were used for univariate analysis. Univariate and multivariate stepwise binary logistic regression analyses were utilized for assessing independent predictors of multiple vessel disease, while univariate and multivariate stepwise Cox proportional hazards regression analyses were adopted for identifying independent predictors of long-term mortality. Kaplan-Meier analysis was used for determining event-free survival. Statistical analysis was performed using SPSS statistical software for Windows version 13 (SPSS for Windows, version 13; SPSS Inc., IL, USA). A two-sided *P* value less than 0.05 was considered to be statistically significant.

RESULTS

Baseline characteristics, angiographic findings, and incidence of coronary artery intervention of 1,305 study patients (Table I):

The BMI and the incidence of previous stroke did not differ among group 1 (multivessel disease), group 2 (single-vessel disease), and group 3 (normal insignificant coronary artery disease). There was no difference in age between groups 2 and 3, however, group 1 was significantly older than groups 2 and 3. The incidences of male gender and previous myocardial infarction were similar between groups 1 and 2, but these two parameters were higher in these two groups than in group 3.

The incidence of current smoking did not differ between groups 1 and 2 and it showed no difference in group 1 as compared to group 3. However, it was significantly higher in group 2 than in group 3. The incidences of diabetes mellitus and hypertension were remarkably higher in group 1 than in groups 2 and 3, but there were no differences between groups 2 and 3. Although the serum total cholesterol level was similar among the 3 groups, the incidence of statin therapy was notably higher in groups 1 and 2 than in group 3.

The levels of plasma tHcy and fasting blood sugar did not differ between groups 2 and 3, but they were significantly lower in groups 2 and 3 than in group 1. Furthermore, the serum

Table I. Baseline Characteristics, Angiographic Results, and Incidence of Coronary Intervention of 1,305 Patients

Variable	Group 1* (n = 676)	Group 2* (n = 367)	Group 3* (n = 262)	P
Age (years)	63.6 $\pm$ 10.0 ^a	61.7 $\pm$ 10.4 ^b	61.5 $\pm$ 10.2 ^b	0.002
Male gender (%)	75.6% (511) ^a	78.5% (288) ^a	66.0% (173) ^b	0.001
BMI (kg/m^2)	25.9 $\pm$ 3.8	26.2 $\pm$ 3.6	26.5 $\pm$ 4.1	0.112
Current smoking (%)	28.8% (195) ^{ab}	33.8% (124) ^a	23.7% (62) ^b	0.022
Diabetes mellitus (%)	38.3% (259) ^a	26.2% (96) ^b	19.5% (51) ^b	< 0.001
Hypertension (%)	61.7% (414) ^a	52.9% (194) ^b	53.8% (141) ^b	0.014
Statin use (%) [†]	57.4% (388) ^a	53.4% (196) ^a	28.2% (74) ^b	< 0.001
Previous stroke (%)	7.7% (52)	6.5% (24)	6.5% (17)	0.712
Old myocardial infarction (%)	18.8% (127) ^a	21.3% (78) ^a	11.1% (29) ^b	0.003
Homocysteine level ($\mu\text{mol/L}$)	11.55 $\pm$ 4.37 ^a	10.87 $\pm$ 3.99 ^b	10.38 $\pm$ 3.80 ^b	< 0.001
Total cholesterol level (mg/dL)	184.2 $\pm$ 42.6	179.2 $\pm$ 36.3	178.5 $\pm$ 36.5	0.057
Fasting blood sugar (mg/dL)	146.6 $\pm$ 101.8 ^a	126.6 $\pm$ 51.1 ^b	122.8 $\pm$ 60.3 ^b	< 0.001
WBC ($\times 10^3/\text{mL}$)	7.3 $\pm$ 2.8	7.1 $\pm$ 2.4	7.1 $\pm$ 5.2	0.699
Creatinine (mg/dL)	1.1 $\pm$ 0.5 ^a	1.1 $\pm$ 0.5 ^{ab}	1.0 $\pm$ 0.4 ^b	0.003
Unstable angina (%)	51.8% (350) ^a	46.3% (170) ^a	12.6% (33) ^b	< 0.001
PCI (%)	77.7% (525) ^a	68.1% (250) ^b	0% (0) ^c	< 0.001
CABG (%)	15.4% (104) ^a	3.3% (12) ^b	0% (0) ^c	< 0.001
Long-term mortality (%) [‡]	3.3% (22) ^a	2.7% (10) ^a	0% (0) ^b	0.014
CV death	1.3% (9)	1.1% (4)	0% (0)	
Non-CV death	1.9% (13)	1.6% (6)	0% (0)	

Data are expressed as mean $\pm$ SD or % (n) of patients. *Group 1, multi-vessel disease; Group 2, single-vessel disease; Group 3, normal or insignificant coronary artery disease. [†]Statin use defined as statin therapy ≥ 5 days prior to blood sampling. [‡]Long-term mortality mean follow-up time = 365.9 ± 205.8 days. BMI indicates body mass index; CV, cardiovascular; CABG, coronary artery bypass surgery; PCI, percutaneous coronary intervention; and WBC, white blood cell count. Letters (^{a,b,c}) indicate significant difference (at 0.05 level) by Bonferroni's multiple comparison procedure.

Table II. Impact of Higher Plasma Level of Homocysteine on Angiographic Results, Incidence of Coronary Artery Intervention, and Clinical Outcome of 1,305 Patients

Variable	Higher tHcy ^a (n = 652)	Lower tHcy ^a (n = 653)	P
Age (years)	64.1 ± 10.2	61.2 ± 10.0	< 0.001
Male gender (%)	81.7% (533)	67.2% (439)	< 0.001
BMI (kg/m ²)	26.0 ± 3.9	26.2 ± 3.8	0.592
Current smoking (%)	32.1% (209)	26.3% (172)	0.023
Diabetes mellitus (%)	32.1% (209)	30.2% (197)	0.462
Hypertension (%)	60.1% (392)	54.7% (357)	0.046
Statin use (%) [†]	49.4% (322)	51.5% (336)	0.455
Previous stroke (%)	8.7% (57)	5.5% (36)	0.023
Old myocardial infarction (%)	20.1% (131)	15.8% (103)	0.042
Cholesterol level (mg/dL)	178.3 ± 37.2	184.9 ± 42.0	0.003
Fasting blood sugar (mg/dL)	132.3 ± 59.0	139.9 ± 101.4	0.107
WBC (× 10 ³ /mL)	7.3 ± 3.8	7.1 ± 2.7	0.270
Creatinine (mg/dL)	1.3 ± 0.6	1.0 ± 0.3	< 0.001
Unstable angina (%)	43.3% (282)	41.5% (271)	0.522
Multivessel disease (%) [‡]	57.4% (374)	46.2% (302)	< 0.001
PCI (%)	61.8 (403)	57.6% (376)	0.119
CABG (%)	8.4% (55)	9.3% (61)	0.565
Repeated coronary intervention (%)	4.3% (28)	3.7% (24)	0.568
Long-term mortality (%) [§]	3.4% (22)	1.5% (10)	0.031

Data are expressed as mean ± SD or % (n) of patients. ^aHigher tHcy group defined as > median level (10.29 μmol/L) of total homocysteine (tHcy) and lower tHcy group defined as ≤ median level (10.29 μmol/L) of tHcy.

[†]Statin use defined as statin was used ≥ 5 days prior to blood sampling.

[‡]Multivessel disease defined as ≥ 2 obstructive coronary artery disease.

[§]Long-term mortality mean follow-up time = 365.9 ± 205.8 days. BMI indicates body mass index; CABG, coronary artery bypass surgery; PCI, percutaneous coronary intervention; and WBC, white blood cell count.

Table III. Correlation Between Homocysteine Level and Enrollment Variables in Table I

Variable	R	P [*]
Age	0.145	< 0.001
Male gender	0.191	< 0.001
Current smoking	0.07	0.011
Previous stroke	0.071	0.011
Old myocardial infarction	0.066	0.017
Multivessel disease	0.106	< 0.001
Creatinine level	0.335	< 0.001
Cholesterol level	-0.09	0.001

^{*}P by Pearson's correlation analysis. PCI indicates percutaneous coronary intervention.

level of creatinine was substantially lower in group 3 than in group 1. However, this parameter did not differ between groups 1 and 2 or between groups 2 and 3. Moreover, the white blood cell count was similar among the 3 groups.

The prevalence of unstable angina was markedly higher in groups 1 and 2 than in group 3, but it showed no difference between groups 1 and 2. Group 3 patients did not receive any PCI or CABG. However, the incidences of PCI and CABG were notably higher in group 1 than in groups 2 and 3, and higher in group 2 than in group 3. The cumulative long-term mortality (combined noncardiovascular and cardiovascular death) did not differ between groups 1 and 2, but it was significantly higher in groups 1 and 2 than in group 3.

Table IV. Univariate Binary Logistic Regression Analysis for Multivessel Disease

Variable	Odds Ratio	95% CI	P
Age	1.019	1.008-1.030	0.001
Diabetes mellitus	2.037	1.600-2.592	< 0.001
Hypertension	1.387	1.113-1.728	0.004
Homocysteine level	1.053	1.025-1.082	< 0.001
Creatinine level	1.471	1.118-1.935	0.006
Total cholesterol level	1.003	1.001-1.006	0.017
Fasting blood sugar	1.005	1.003-1.007	< 0.001

CI indicates confidence interval.

Table V. Multivariate Binary Logistic Regression Analysis of Multivessel Disease

Variable	Odds Ratio	95% CI	P
Fasting blood sugar	1.002	1.000-1.004	0.029
Diabetes mellitus	1.761	1.315-2.358	0.001
Homocysteine level	1.038	1.006-1.072	0.019
Age	1.023	1.010-1.037	0.001

CI indicates confidence interval.

Impact of higher plasma level of total homocysteine on angiographic findings, incidence of coronary artery intervention, and clinical outcome of 1,305 patients (Table II): To investigate whether the plasma tHcy level was predictive of the severity of obstructive CAD and long-term clinical outcome, the patients were categorized into a higher tHcy group [defined as those patients with tHcy > median level (10.29 μmol/L)] and a lower tHcy group [defined as those patients with tHcy ≤ median level (10.29 μmol/L)]. The BMI, level of fasting blood sugar, white blood cell count, and the incidences of diabetes mellitus, statin use, unstable angina, PCI, and CABG were similar between the two groups. However, the mean age of patients in the higher tHcy group was higher than that in the lower tHcy group. In addition, the incidences of male gender, hypertension, current smoking, previous stroke, and old myocardial infarction were notably higher in the higher tHcy group compared with those in the lower tHcy group. Moreover, the serum creatinine level was significantly higher, whereas the total cholesterol level was notably lower in the higher tHcy group than in the lower tHcy group. Furthermore, as expected, the incidences of multiple-vessel disease and long-term mortality were remarkably higher in the higher tHcy group than in the lower tHcy group.

Correlation between homocysteine level and enrollment variables (Table III): To assess the correlation between baseline variables and plasma tHcy level, Pearson's correlation analysis was performed in the current study. The results showed that age, male gender, serum creatinine concentration, and multiple-vessel disease were the strongest factors correlated with an increased plasma tHcy concentration. Moreover, current smoking, previous stroke, and old myocardial infarction were also significantly correlated with the plasma tHcy level. On the other hand, a higher total cholesterol level was inversely correlated to an increased plasma level of tHcy.

Binary logistic regression analysis for multiple-vessel disease (Tables IV and V): To determine whether the plasma level of

Table VI. Univariate Cox Regression Analysis on Long-Term Mortality

Variable	Hazard Ratio	95% CI	P
Age	1.078	1.036-1.121	< 0.001
Body mass index	0.880	0.815-0.951	0.001
Previous stroke	2.885	1.187-7.011	0.019
Serum creatinine	1.526	1.204-1.934	0.001
Fasting blood sugar	1.003	1.001-1.004	< 0.001
Homocysteine level	2.172	1.027-4.590	0.042

CI indicates confidence interval.

Table VII. Multivariate Cox Regression Analysis on Long-Term Mortality

Variable	Hazard Ratio	95% CI	P
Fasting blood sugar	1.003	1.001-1.005	0.001
Serum creatinine	2.057	1.351-3.133	0.001
Body mass index	0.839	0.746-0.942	0.003
Age	1.058	1.012-1.106	0.012

CI indicates confidence interval.

tHcy was independently predictive of multiple-vessel disease, univariate and multiple binary logistic regression analyses were performed. Univariate analysis revealed that plasma level of tHcy, diabetes mellitus, and fasting blood sugar were most strongly predictive of multiple-vessel disease. In addition, age, hypertension, and serum levels of creatinine and total cholesterol were also significantly predictive of multiple-vessel disease.

Multivariate binary logistic regression analysis showed that both age and diabetes mellitus were the most strongly independent predictors of multiple-vessel disease. Furthermore, plasma homocysteine level and fasting blood sugar were also significantly and independently predictive of multiple-vessel disease.

Kaplan-Meier and Cox regression analysis for long-term mortality (Tables VI and VII): This study was designed to determine whether the plasma level of tHcy was independently predictive of long-term mortality. Univariate Cox regression analysis for long-term mortality demonstrated that age, BMI, previous stroke, fasting blood sugar, and serum creatinine levels were remarkably predictive of long-term mortality. However, the plasma level of tHcy was weakly but significantly correlated with long-term mortality.

Multivariate Cox regression analysis for long-term mortality demonstrated that only age, BMI, fasting blood sugar, and serum creatinine levels were significantly and independently predictive of long-term mortality.

Kaplan-Meier analysis (Figure) for event-free survival showed a significantly lower long-term survival rate in patients with a higher plasma tHcy concentration compared with those with a lower plasma tHcy level.

DISCUSSION

This study, which investigated the impact of the plasma level of tHcy on patients with angina pectoris undergoing coronary angiographic study, provided several striking implications. First, a higher plasma level of tHcy was a significant in-

Figure. Kaplan-Meier analysis of free-survival rate. Higher tHcy group: Plasma tHcy > 10.29 $\mu\text{mol/L}$; Lower tHcy group: Plasma tHcy $\leq 10.29 \mu\text{mol/L}$.

dependent predictor of multiple vessel disease. Second, the plasma level of tHcy was not an independent predictor of long-term mortality.

Similar to CRP,¹¹⁻¹³⁾ the plasma total tHcy level has been shown to be a new risk factor for CAD.^{14,15,20)} Regardless of the underlying etiology or mechanisms, plasma tHcy has been revealed to be strongly correlated with obstructive CAD,^{7,20)} restenosis, major adverse cardiac events after percutaneous coronary angioplasty,²¹⁾ and all-cause mortality.^{22,23)} Additionally, the association between increased plasma tHcy level and the severity of atherosclerosis has been investigated.¹⁸⁾ Furthermore, the link between increased plasma tHcy level and progression of atherosclerosis has been previously reported.²⁴⁾ Still another study has even shown that plasma tHcy level predicts mortality independent of traditional risk factors and CRP levels in patients with angiographically defined coronary artery disease.²⁵⁾ One of the important findings in the current study is that the plasma tHcy level was remarkably higher in patients with significant obstructive CAD than in those without significant obstruction. The most important finding in the present study is that multivariate binary logistic regression analysis demonstrated that plasma tHcy was independently predictive of significantly obstructive CAD in patients with experience of angina pectoris. Interestingly, the majority of these patients who had significant obstruction of CAD received PCI. The results of the present study, therefore, are consistent with those of previous studies.^{7,14-21)}

Interestingly, despite the consistency of the finding in the current study that links plasma level of tHcy with CAD, the impact of plasma tHcy level on long-term mortality was unremarkable after multivariate Cox regression analysis. In this way, our findings corroborated those of previous studies showing that plasma tHcy is not independently predictive of long-term clinical outcome.^{7,16,26)} In contrast to our findings and also those of previous studies,^{7,16,26)} some previous investigations have previously shown that the plasma level of tHcy is a significant predictor of long-term outcome regardless of the disease entity.^{22,23,27)} The cause of the discrepancy between previous findings and those from our and other studies remains

uncertain. Possible causes are as follows: First, plasma tHcy level has been shown to be associated with both genetic and nutritional factors, as well as dietary habits.²⁸⁾ Second, an increased plasma level of tHcy can be found in subjects with chronic alcoholic consumption, impairment of liver function, and those with altered methionine and homocysteine metabolism.²⁹⁾ Third, the plasma level of tHcy has been shown to be strongly correlated with age, gender, smoking, serum folate, vitamin B12, and renal function.^{22,30-32)} Interestingly, in the present study, we also found that age, gender, current smoking, and the serum level of creatinine were strongly correlated with plasma tHcy level. Accordingly, the findings of the current investigation corroborate those of previous studies,³⁰⁻³²⁾ and further confirm that an increased plasma level of tHcy is multifactorial.^{22,30-33)} Finally, methodological problems have been reported to contribute to uncertain determination of the strength of these relationships, especially those among an elevated plasma tHcy level and traditional risk factors.³⁴⁾

Undoubtedly, traditional CAD risk factors have been recognized as independent predictors of long-term untoward clinical outcomes in study subjects with or without chest pain syndrome.³⁵⁻³⁸⁾ With the recruitment of a large patient population, the present study further shows that the plasma level of tHcy, age, BMI, fasting blood sugar, and creatinine levels were independently predictive of long-term mortality. Therefore, our findings reinforce those of previous studies.³⁵⁻³⁸⁾

This study has several limitations. First, although it demonstrates that plasma tHcy was an independent predictor of obstructive CAD, the mechanism underlying the role of plasma tHcy in endothelial dysfunction and propagation of coronary atherosclerosis and obstruction remains uncertain. Second, the endpoint of this study was designed for a clinical follow-up rather than routinely for an angiographic follow-up. Therefore, the number of patients who required repeated coronary intervention may be underestimated. Finally, only the correlation between the incidence of unstable angina rather than the plaque instability and plasma tHcy level was investigated.

In conclusion, plasma level of tHcy was independently predictive of MVD but it was not an independent predictor of long-term outcome in patients with angina pectoris undergoing coronary angiographic study.

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