

Prognostic Value of Elevated Homocysteine Levels in Korean Patients with Coronary Artery Disease: A Propensity Score Matched Analysis

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Background and Objectives: We sought to determine whether an elevated homocysteine (Hcy) level is associated with a worse prognosis in Korean patients with coronary artery disease (CAD).

Subjects and Methods: A total of 5839 patients (60.4% male, mean age 61.3±11.2 years) with CAD were enrolled from 2000 to 2010 at Gangnam Severance Hospital. CAD was diagnosed by invasive coronary angiography. Laboratory values including Hcy level were obtained on the day of coronary angiography and analyses were performed shortly after sampling. Patients were divided into two groups according to their Hcy levels. Baseline risk factors, coronary angiographic findings, length of follow-up, and composite endpoints including cardiac death (CD) and non-fatal myocardial infarction (NFMi) were recorded. 1:1 propensity score matched analysis was also performed.

Results: Over a mean follow-up period of 4.4±2.5 years, there were 132 composite endpoints (75 CD and 57 NFMi) with an event rate of 2.3%. Mean Hcy level was 9.9±4.3 μmol/L (normal Hcy 7.9±1.5 μmol/L and elevated Hcy 13.9±5.1 μmol/L). Kaplan-Meier survival analysis showed an association of elevated Hcy level with worse prognosis (p<0.0001). In addition, a multivariate Cox regression analysis showed an association of elevated Hcy level with worse prognosis for both the entire cohort (hazard ratio [HR] 2.077, 95% confidence interval [CI] 1.467-2.941, p<0.0001) and the propensity score matched cohort (HR 1.982, 95% CI 1.305-3.009, p=0.001).

Conclusion: Elevated Hcy level is associated with worse outcomes in Korean patients with CAD. (*Korean Circ J* 2016;46(2):154-160)

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Introduction

Homocysteine (Hcy) is a sulfur-containing amino acid metabolized either by the remethylation pathway to methionine or the trans-sulfuration pathway to cysteine; each pathway depends on other series of biochemical enzymes such as methionine synthetase and methylene tetrahydrofolate reductase as well as vitamin B12 and folic acid. A later pathway is dependent on the enzymes cystathionine β-synthetase and pyridoxine (vitamin B6). Any loss of one of those pathways and/or a dietary deficiency can result in an elevated Hcy level.¹⁾ Therefore, Hcy level may be influenced by factors such as nutrition, genetics, lifestyle, ethnicity, age,

and gender.^{1,2)} Epidemiological studies have demonstrated that progression of atherosclerosis plaques is correlated with elevated circulating Hcy due to increased thrombogenicity, oxidative stress status and endothelial dysfunction.^{3,4)} Consequently, a recent large prospective study reported an independent association of elevated Hcy level with coronary artery disease (CAD), and the prognostic value of elevated Hcy level is well validated in Caucasians, both in the general population and in the patients with CAD.⁵⁻⁹⁾ However, there are limited data regarding the prognostic value of elevated Hcy in other ethnicities. Because Hcy level may be influenced by ethnicity, it is not certain whether elevated Hcy level is associated with worse outcome in Korean patients with CAD. Therefore, we sought to evaluate the prognostic value of elevated Hcy level in such Korean patients.

Subjects and Methods

Study design and patient selection

A total of 5839 patients (60.4% male, mean age 61.3±11.2 years) with CAD from 2000 to 2010 at Gangnam Severance Hospital were enrolled. CAD was diagnosed by invasive coronary angiography. Laboratory values including Hcy level were obtained on the day of coronary angiography and analyses were performed shortly after sampling. Patients were divided into two groups according to their Hcy levels. Baseline risk factors, coronary angiographic findings, length of follow-up, and composite endpoints including cardiac death (CD) and non-fatal myocardial infarction (NFMI) were recorded. Institutional review committee approval and informed consent were obtained.

Definition of variables

Risk factors were recorded as categorical variables in all patients. Hypertension was defined as systolic blood pressure≥140 mmHg and/or diastolic blood pressure≥90 mmHg, or by antihypertensive prescription. Type 2 diabetes was defined by hypoglycemic agents or insulin prescription, fasting plasma glucose≥126 mg/dL, glycosylated hemoglobin≥6.5%, or known but untreated hyperglycemia. Hyperlipidemia was defined by total cholesterol≥240 mg/dL, triglycerides≥200 mg/dL, low-density lipoprotein cholesterol≥130 mg/dL, high-density lipoprotein cholesterol<40 mg/dL, and/or by lipid-lowering prescription. A patient was considered a smoker, if the patient currently smoked or had smoked up until 1 month prior to the baseline coronary angiography. Non-obstructive CAD was defined as <50% luminal narrowing, whereas obstructive CAD was defined as ≥50% luminal narrowing. The extent of obstructive CAD was categorized according to the number of vessels involved (1, 2, or 3).

Laboratory measurements

Total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol were measured directly using colorimetric and turbidimetric assays with an autoanalyzer AU5800 (Beckman Coulter, Irvine, CA, USA). Hcy measurement was determined using a commercially available enzymatic colorimetric assay AU5800 (Beckman Coulter, Irvine, CA, USA), and the values were expressed in μmol/L. Patients were divided into two groups according to the respective Hcy concentration levels: 1) normal Hcy group (lower two tertiles [Hcy median value 7.9 μmol/L, range: 0.1-10.3 μmol/L]) and 2) elevated Hcy group (upper tertile [Hcy median value 12.5 μmol/L, range: 10.4-94.0 μmol/L]).

Endpoint determination and follow-up data acquisition

Composite endpoints were defined as CD or NFMI. To avoid treatment bias, coronary artery revascularization procedures, such as coronary artery bypass graft surgery and percutaneous coronary intervention, were not regarded as endpoints.¹⁰⁾ For patients with multiple cardiovascular events, only the first event was considered for analysis. Cardiac death was defined as death caused by acute myocardial infarction, ventricular arrhythmias, refractory heart failure, or cardiogenic shock. Diagnosis of NFMI required the presence of at least two of the three following criteria: characteristic chest pain, elevated cardiac enzymes, or electrocardiographic alterations indicative of myocardial infarction. Patient follow-up data were collected using electronic medical record reviews and/or standardized telephone interviews.

Statistical analysis

Continuous data were expressed as a mean value±standard deviation. All categorical data were presented as a percentage or an absolute number. Analyses of continuous data were performed using independent t-test and analyses of categorical data were performed using chi-square test to assess differences between the two groups. The C-statistic for the logistic regression model used for calculation of the propensity score matching for the 2 groups was 0.66. Patients with elevated Hcy level were then 1-to-1 matched to the patients with normal Hcy level on the propensity scores using the nearest available pair matching method. The procedure yielded 1818 well-matched pairs. Cumulative event rates as a function over time were estimated using the Kaplan-Meier method, and survival curves of composite CD/NFMI were compared using the log-rank test for both the entire cohort and the propensity score matched cohort. Univariate and multivariate Cox regression analyses were performed to determine associations between clinical characteristics (age, gender, hypertension, smoking, and hyperlipidemia), lipid parameters, Hcy, extent of CAD

Table 1. Baseline clinical characteristics according to Hcy levels

	Total cohort (n=5839)			Propensity score matched cohort (n=3636)		
	Normal Hcy	Elevated Hcy	p	Normal Hcy	Elevated Hcy	p
Age (years)	59.9±10.8	63.9±11.5	<0.0001	62.7±10.2	63.3±11.4	0.111
Male gender	2109/3886 (54.3)	1416/1953 (72.5)	<0.0001	1318/1818 (72.5)	1298/1818 (71.4)	0.460
Diabetes	854/3886 (22.0)	497/1953 (25.4)	0.003	465/1818 (25.6)	458/1818 (25.2)	0.790
Hypertension	1998/3886 (51.4)	1140/1953 (58.4)	<0.0001	1045/1818 (57.5)	1052/1818 (57.9)	0.814
Smoking	1046/3886 (26.9)	669/1953 (34.3)	<0.0001	636/1818 (35.0)	614/1818 (33.8)	0.442
Hyperlipidemia	2508/3886 (64.5)	1372/1953 (70.3)	<0.0001	1260/1818 (69.3)	1273/1818 (70.0)	0.639
Clinical diagnosis			<0.0001			0.653
Silent IHD	611/3886 (15.7)	398/1953 (20.4)		349/1818 (19.2)	354/1818 (19.5)	
Stable angina	1740/3886 (44.8)	836/1953 (42.8)		825/1818 (45.4)	782/1818 (43.0)	
Unstable angina	1090/3886 (28.0)	510/1953 (26.1)		453/1818 (24.9)	485/1818 (26.7)	
NSTEMI	216/3886 (5.6)	114/1953 (5.8)		101/1818 (5.6)	107/1818 (5.9)	
STEMI	229/3886 (5.9)	95/1953 (4.9)		90/1818 (5.0)	90/1818 (5.0)	

Data are expressed as number (%) or mean±standard deviation. Hcy: homocysteine, IHD: ischemic heart disease, NSTEMI: non-ST elevation myocardial infarction, STEMI: ST elevation myocardial infarction

and clinical outcome in both the entire cohort and the propensity score-matched cohort. Hazard ratios (HRs) were calculated as an estimate of the risk associated with a particular variable with 95% confidence intervals (CIs), based on binomial distributions. All analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL, USA). A p of less than 0.05 was considered statistically significant.

Results

Of the 5839 patients in the study, 60.4% were male and the mean age was 61.3±11.2 years. Mean Hcy level was 9.9±4.3 µmol/L (7.9±1.5 µmol/L for normal Hcy groups and 13.9±5.1 µmol/L for elevated Hcy group, respectively). A summary of the baseline characteristics of both the entire cohort and propensity matched cohort according to the Hcy level is shown in Table 1. Patients with elevated Hcy levels tended to be older (p<0.0001), male (p<0.0001), diabetic (p=0.003), hypertensive (p<0.0001), smoker (p<0.0001), and hyperlipidemic (p<0.0001) (Table 1). More patients with elevated Hcy level had acute coronary syndrome at their clinical presentation (p<0.0001) (Table 1). A summary of laboratory and angiographic characteristics of both the entire cohort and the propensity matched cohort according to the Hcy level is shown in Table 2. Patients with elevated Hcy level had higher triglyceride levels (p=0.020) and lower high-density lipoprotein cholesterol levels (p<0.0001) (Table 2). In addition, elevated Hcy level showed an association with higher rates of both obstructive CAD (p<0.0001) and 3-vessel obstructive CAD (p<0.0001), at the time of baseline

coronary angiography (Table 2). Consequently, higher coronary revascularization rates as well as coronary bypass graft surgery rates were observed in patients with elevated Hcy level (Table 2).

Clinical outcomes

Over a mean follow-up period of 4.4±2.5 years, there were 132 composite endpoints (75 CD and 57 NFMI) with an event rate of 2.3%. The composite CD/NFMI rates were 1.5% (60/3886) and 3.7% (72/1953) for the normal Hcy and the elevated Hcy group, respectively, which were significantly different (p<0.0001) (Fig. 1A). In multivariate Cox proportional survival analysis, age (HR 1.054, 95% CI 1.035-1.073, p<0.0001), diabetes (HR 2.137, 95% CI 1.052-3.039, p<0.0001), obstructive CAD (HR 3.226, 95% CI 1.721-6.047, p<0.0001), and elevated Hcy (HR 2.077, 95% CI 1.467-2.941, p<0.0001) were independent risk factors for composite CD/NFMI (Table 3A).

Propensity score matched analysis

After performing propensity score matching, a total of 1818 matched pairs (1818 patients from the normal Hcy group and 1818 patients from the elevated Hcy group) were generated. There were no significant differences in baseline, laboratory and angiographic characteristics for the propensity score matched subjects except for Hcy levels (Tables 1 and 2). A total of 97 composite endpoints (56 CD and 41 NFMI) occurred during the follow-up period in the matched cohorts. The respective composite CD/NFMI rates were 1.9% (34/1818) and 3.5% (63/1818) for normal Hcy and elevated Hcy group. The elevated Hcy group was associated with

Table 2. Laboratory and angiographic characteristics according to Hcy levels

	Total cohort (n=5839)			Propensity score matched cohort (n=3636)		
	Normal Hcy	Elevated Hcy	p	Normal Hcy	Elevated Hcy	p
Total cholesterol (mg/dL)	171.6±37.4	169.9±40.0	0.139	170.4±37.8	170.5±40.0	0.934
Triglycerides (mg/dL)	135.6±84.2	141.0±83.4	0.020	138.6±79.3	140.9±83.0	0.397
LDL-C (mg/dL)	105.9±32.3	106.0±34.1	0.938	106.2±32.3	106.1±34.2	0.948
HDL-C (mg/dL)	44.7±11.4	42.3±11.4	<0.0001	43.0±10.5	42.6±11.5	0.334
Hcy (μmol/L)	7.9±1.5	13.9±5.1	<0.0001	8.1±1.4	13.8±4.8	<0.0001
Obstructive CAD	2404/3886 (61.9)	1366/1953 (69.9)	<0.0001	1263/1818 (69.5)	1258/1818 (69.2)	0.857
Extent of CAD			<0.0001			0.339
Non-obstructive CAD	1482/3886 (38.1)	587/1953 (30.1)		555/1818 (30.5)	560/1818 (30.8)	
1VD	1005/3886 (25.9)	449/1953 (23.0)		443/1818 (24.4)	422/1818 (23.2)	
2VD	699/3886 (18.0)	375/1953 (19.2)		378/1818 (20.8)	352/1818 (19.4)	
3VD	700/3886 (18.0)	542/1953 (27.8)		442/1818 (24.3)	484/1818 (26.6)	
Revascularization	1764/3886 (45.4)	996/1953 (51.0)	<0.0001	946/1818 (52.0)	924/1818 (50.8)	0.465
BMS	509/3886 (13.1)	256/1953 (13.1)		259/1818 (14.2)	240/1818 (13.2)	
DES	1041/3886 (26.8)	554/1953 (28.4)		547/1818 (30.1)	512/1818 (28.2)	
CABG	214/3886 (5.5)	186/3886 (9.5)		140/1818 (7.7)	172/1818 (9.5)	

Data are expressed as number (%) or mean±standard deviation. Hcy: homocysteine, LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, CAD: coronary artery disease, VD: vessel disease, BMS: bare metal stent, DES: drug-eluting stent, CABG: coronary artery bypass graft

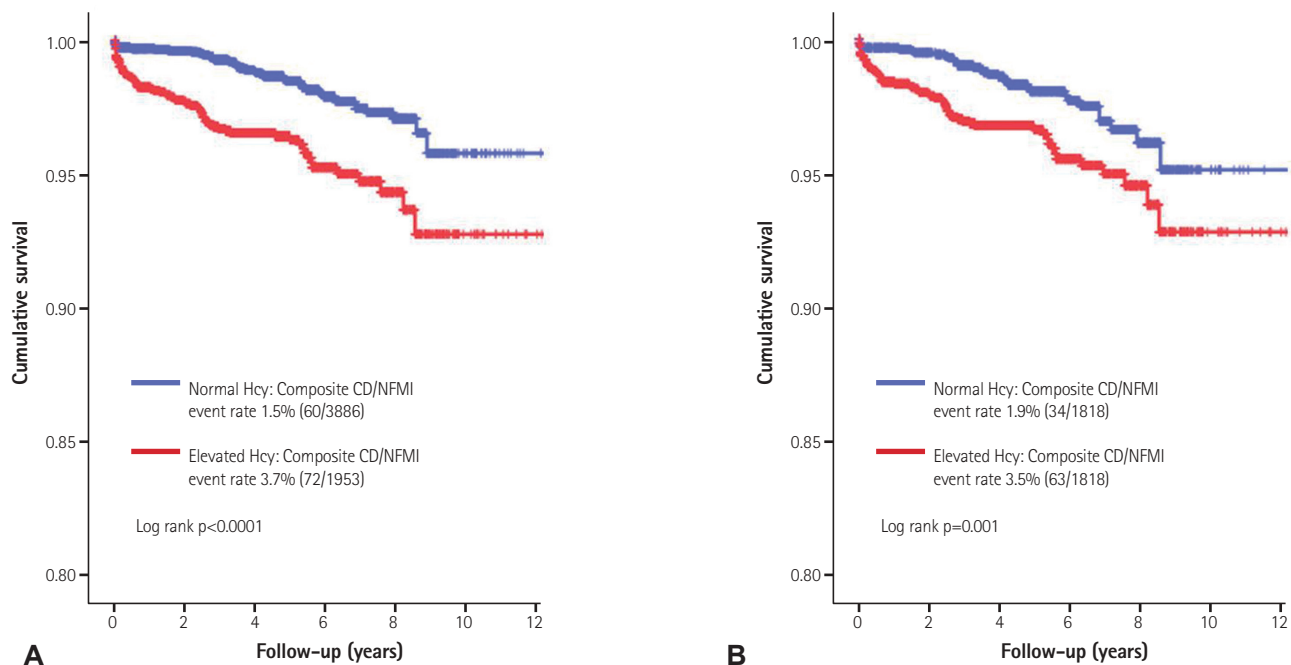


Fig. 1. Kaplan-Meier survival analysis according to Hcy levels. (A) Total cohort and (B) propensity score matched cohort. Hcy: homocysteine. CD: cardiac death, NFMI: non-fatal myocardial infarction.

Table 3. Univariate and multivariate Cox regression analysis for predicting composite CD/NFMI in (a) total cohort and (b) propensity score matched cohort

(A) Total cohort

	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Age	1.068	1.049-1.087	<0.0001	1.054	1.035-1.073	<0.0001
Male gender	1.238	0.861-1.779	0.249			
Diabetes	2.623	1.858-3.703	<0.0001	2.137	1.502-3.039	<0.0001
Hypertension	1.480	1.036-2.116	0.031	1.006	0.697-1.452	0.973
Smoking	1.090	0.759-1.563	0.641			
Hyperlipidemia	1.280	0.869-1.884	0.212			
Obstructive CAD	3.853	2.212-6.713	<0.0001	3.226	1.721-6.047	<0.0001
Extent of CAD			<0.0001			
Non-obstructive CAD	1.000	(reference)				
1VD	2.160	1.122-4.159	0.021			
2VD	2.438	1.243-4.781	0.010			
3VD	7.017	3.951-12.460	<0.0001			
Hcy	2.571	1.824-3.622	<0.0001	2.077	1.467-2.941	<0.0001
Revascularization	1.534	1.075-2.188	0.018	0.766	0.514-1.141	0.190

(B) Propensity score matched cohort

	Univariate			Multivariate		
	HR	95% CI	p	HR	95% CI	p
Age	1.059	1.037-1.082	<0.0001	1.053	1.030-1.076	<0.0001
Male gender	0.773	0.504-1.185	0.237			
Diabetes	2.695	1.808-4.019	<0.0001	2.360	1.580-3.527	<0.0001
Hypertension	1.273	0.841-1.927	0.253			
Smoking	0.943	0.623-1.429	0.783			
Hyperlipidemia	1.232	0.776-1.955	0.376			
Obstructive CAD	3.301	1.714-6.358	<0.0001	2.499	1.289-4.844	0.007
Extent of CAD			<0.0001			
Non-obstructive CAD	1.000	(reference)				
1VD	2.029	0.936-4.398	0.073			
2VD	1.995	0.896-4.442	0.091			
3VD	5.461	2.777-10.738	<0.0001			
Hcy	2.001	1.318-3.038	0.001	1.982	1.305-3.009	0.001
Revascularization	1.328	0.878-2.008	0.179			

CD: cardiac death, NFMI: non-fatal myocardial infarction, HR: hazard ratio, CI: confidence interval, CAD: coronary artery disease, VD: vessel disease, Hcy: homocysteine

significantly worse outcomes than the normal Hcy group in the matched cohort ($p=0.001$) (Fig. 1B). In addition, in the multivariate analysis, the elevated Hcy group still had almost 2-fold higher risk for composite CD/NFMI, when compared to the normal Hcy group (HR 1.982, 95% CI 1.305-3.009, $p=0.001$) (Table 3B).

Discussion

Hcy is an intermediate metabolite of methionine that contributes to atherosclerosis through several mechanisms, such as endothelial dysfunction, increased permeability of lipid and inflammatory

cells, lipoprotein oxidation, vascular inflammation, smooth muscle proliferation, platelet activation, and abnormalities in the clotting cascade.¹¹⁻¹³ During the process of Hcy metabolism pathways, any interference of one of those pathways and/or a dietary deficiency can result in elevation of Hcy level.¹¹ Therefore, Hcy level may be influenced by factors such as nutrition, genetics, lifestyle, ethnicity, age, and gender.¹¹ Previous study by Cappuccio et al.² reported the discordance of Hcy levels which differ among Caucasians, African origins, and South Asians living in England, suggested a variance of the Hcy level between ethnicities. Another study reported that the frequency of the homozygous dominant mutation genotype of the enzyme methylene tetrahydrofolate reductase (C677T gene) is higher in the Hispanic population in the United States compared to other populations.¹⁴ Since, genetic polymorphism of methylene tetrahydrofolate reductase affects the Hcy level, the ethnic differences of this enzyme polymorphism may result in variations in Hcy level.

A recent large prospective cohort study reported an association of elevated Hcy level with the severity as well as the presence of CAD.⁵ This study confirmed this association even after correction for baseline differences, suggesting an independent role of Hcy in prediction of CAD risk. In addition, since the Hcy level may be influenced by ethnicity, several previous cross-sectional studies from non-Caucasian populations reported the association between Hcy level and CAD. Baños-González et al.¹⁵ reported an association of elevated Hcy with CAD in a Hispanic male population. Another study by Ghassibe-Sabbagh et al.¹⁶ reported an association of elevated Hcy with CAD in a Middle East Asian population. Kumakura et al.¹⁷ reported an association of elevated Hcy with CAD in Japanese patients with peripheral artery disease. Consistent with these studies, our study demonstrated that elevated Hcy levels have positive correlation with both the presence and extent of CAD in the Korean population.

Previous meta-analysis study by Homocysteine Studies Collaboration consisting of 30 prospective or retrospective studies reported that elevated Hcy is a modest independent predictor of ischemic heart disease in a healthy population.⁶ In addition, a recent study suggested that elevated Hcy level has additive prognostic value in terms of predicting adverse cardiac events in the general population.⁷ With regard to the prognostic value of elevated Hcy level in other ethnic general populations, Sun et al.¹⁸ reported a significant association of elevated Hcy level with cardiovascular events and all cause mortality in the general Chinese population. The prognostic value of elevated Hcy in patients with CAD is still a subject under debate. Previous study reported that elevated Hcy level was not associated with worse prognosis in patients with CAD.¹⁹ In contrast, other study reported that elevated Hcy level

was a strong predictor of mortality in patients with CAD.⁹ Another study reported an association of elevated Hcy level with adverse cardiac events in patients with CAD.⁹ However, aforementioned studies on the prognostic value of elevated Hcy level in patients with CAD were conducted in Caucasians. In the context of these conflicting findings, we evaluated the prognostic value of elevated Hcy level in non-Caucasian (Korean) patients with CAD. In our study, we demonstrated the distribution of Hcy level in Korean patients with CAD. Besides, we documented that elevated Hcy level is associated with worse outcome in Korean patients with CAD, even after propensity score matching for other confounding variables. Therefore, we believe that our data support the clinical value of determining elevated Hcy level in Korean patients with CAD.

There are several limitations in our study. First, this was an observational study conducted at a single center. Second, there are no data for vitamin cofactors (vitamin B6 and B12 and folic acid) required for Hcy metabolism, all of which may affect the Hcy levels. Pharmacological interferences such as vitamin B/folate intake administration or some genetic mutations might have played a role in modifying Hcy levels.²⁰ However, several randomized controlled trials and a recent meta-analysis have shown that Hcy-lowering interventions with vitamin B/folate supplementation did not reduce the occurrence of cardiovascular events, particularly coronary heart disease.²¹⁻²⁶ In conclusion, an elevated Hcy level is associated with worse outcomes in Korean patients with CAD.

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