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Original article

Prognostic impact of homocysteine levels and homocysteine thiolactonase activity on long-term clinical outcomes in patients undergoing percutaneous coronary intervention

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ABSTRACT

Background: Numerous studies have reported the relationship between elevated homocysteine (Hcy) levels and the risk of coronary artery disease. However, there is insufficient information about the effects of Hcy levels on long-term clinical outcomes in patients with coronary artery disease undergoing percutaneous coronary intervention (PCI).

Methods: In the Juntendo-registry cohort from 2003 to 2004, pre-procedural Hcy levels and Hcy thiolactonase activity (HTlase) were measured in 315 consecutive all-comer patients who underwent PCI for stable coronary artery disease or acute coronary syndrome (ACS). Receiver-operating characteristic (ROC) curves were created to assess the optimal cut-off values of Hcy and HTlase. Multivariable Cox proportional hazard regression analysis was used to identify the predictors of clinical outcome. The primary endpoint was all-cause mortality.

Results: The patients' mean age was 66 ± 9 years, and 82.5% were males. The median follow-up period was 10.5 years, and overall mortality was 24.5% (73 deaths). On ROC analysis, the optimal cut-off values of Hcy and HTlase were 13.5 μmol/L and 230 IU/L, respectively. Kaplan–Meier survival analysis showed associations of both higher Hcy levels and lower HTlase activity with worse prognosis (both log-rank $p < 0.001$). On multivariate Cox proportional hazard regression analysis, higher Hcy was strongly associated with the primary outcome, and the adjusted hazard ratio was 3.3 (95% confidence interval, 1.8–5.6; $p < 0.001$).

Conclusions: Pre-procedural high Hcy levels and low HTlase activity were associated with worse long-term mortality in Japanese patients undergoing PCI. Moreover, Hcy levels are strongly predictive for mortality, independent of traditional risk factors. This may have implications for risk stratification and the therapeutic approach in this PCI era.

Introduction

Homocysteine (Hcy) is a sulfhydryl amino acid compound that is considered an intermediate compound in the metabolism of the amino acid methionine into cysteine [1]. There are two pathways that determine the fate of Hcy once formed according to the amount of methionine present: either in the direction of back to methionine with the help of vitamin B12 and folate in cases of deficient methionine, or in the direction of cysteine with the help of vitamin B6 in cases of excess methionine. Deficiencies in compounds such as vitamin B6, vitamin B12, or folate, or enzymes involved in Hcy metabolism lead to varying degrees of hyperhomocysteinemia [2].

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It is estimated that around 20% of cases of coronary artery disease (CAD) cannot be explained based on the traditional Framingham risk factors. Hence came the need to look for other factors that increase the risk of CAD [3]. The association between Hcy and CAD came into focus with the observation that 50% of young patients with homocysteinuria died at an early age from cardiovascular complications [4]. It has also been found that the homozygous mutation of cystathionine-β-synthase can increase Hcy levels up to 40 times the normal levels, causing vascular events such as stroke or myocardial infarction in about 50% of these patients before the age of 30 years [5]. Many studies, either observational or randomized, have shown a strong link between elevated levels of Hcy and an increased risk of CAD, confirming that for each 5 μmol increase in the Hcy level there is an equivalent increase of 19 mg/dl in total cholesterol, with a 20% increase in the risk of CAD [6–12]. However, despite the positive evidence for the correlation between Hcy and CAD, most intervention trials involving treatment with vitamin B and folate have failed to show benefit either for primary or secondary prevention.

Generally, few studies have been done to study the impact of the Hcy level on outcomes after percutaneous coronary intervention (PCI), especially in Asian populations. Thus, this study aimed to evaluate the impact of Hcy levels on survival after PCI in the Japanese population.

Methods

Study subjects

Previously collected demographic data and information about coronary risk factors and medications derived from a single center, prospective, observational study of consecutive patients with CAD who underwent PCI between March 2003 and December 2004 were analyzed.

Blood samples were collected in the early morning after an overnight fast, and blood pressure (BP) was measured at the time of admission. Patients with BP ≥140/90 mmHg or on antihypertensive medication were considered hypertensive. Dyslipidemia was defined as low-density lipoprotein cholesterol (LDL-C) ≥140 mg/dl, high-density lipoprotein cholesterol (HDL-C) ≤40 mg/dl, triglycerides ≥150 mg/dl, and treatment with statins and/or lipid-lowering agents [13]. Diabetes mellitus (DM) was defined as either hemoglobin A1c (HbA1c) ≥6.5% or treatment with insulin or oral hypoglycemic drugs. The estimated HbA1c (%) was calculated as the National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) using the formula HbA1c (%) = 1.02 × HbA1c (JDS; %) + 0.25% [14]. Renal impairment was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m^2, and eGFR was calculated based on the Modification of Diet in Renal Disease Equation modified with a Japanese coefficient using the baseline serum creatinine [15].

Written, informed consent was obtained from all patients. This study proceeded with the approval of our institutional review board in accordance with the Declaration of Helsinki.

Determination of biomarkers

The concentration of Hcy and the activity of homocysteine thiolactonase (HTlase) were determined using commercial kits: Alfresa Auto Hcy Kit (Alfresa Pharma, Osaka, Japan) and Alfresa Auto HTLase (Alfresa Pharma), respectively. These measurements were performed using an automatic analyzer JCA-BM8040 (JEOL, Tokyo, Japan) [16,17]. Other biochemical parameters were measured using routine laboratory methods at our institution.

Outcome measurements

The primary outcome was all-cause death. Clinical follow-up comprised analyses of office visit charts and responses to questionnaires sent to patients or their families and telephone contacts. Mortality data were collected from the medical records of patients who died or who were treated at our institution, and details and causes of death were obtained from other hospitals where patients had been admitted.

Statistical analysis

Continuous variables are reported as means ± standard deviation or medians with 25th and 75th percentiles. Categorical variables are reported as percentages. Comparisons of baseline characteristics were performed with the *t*-test or the Mann–Whitney U test for continuous variables and Pearson's Chi-square test for categorical variables. In order to assess the optimal cut-off values of Hcy and HTlase activity for predicting the primary outcome, receiver-operating characteristic (ROC) curves were created. The optimal cut-off value was defined as the concentration with the highest sum of sensitivity and specificity. On ROC analysis, the optimal cut-off values of Hcy and HTlase activity were 13.5 μmol/L and 230 IU/L, respectively. At these values, Hcy had a sensitivity and specificity of 42% and 86%, respectively, and HTlase activity had sensitivity and specificity of 32% and 85%, respectively. The areas under the ROC curves of Hcy and HTlase activity were 0.63 and 0.58, respectively. Event-free survival was analyzed with the Kaplan–Meier method, and the curves were compared with the log-rank test.

The effects of Hcy levels and HTlase activity on death after PCI were determined using multivariable Cox proportional hazard regression analysis. Age, sex male, body mass index (BMI), hypertension, diabetes, current smoking, family history of CAD, chronic kidney disease (CKD), left ventricular ejection fraction (LVEF), triple-vessel disease, stent size, stent length, and medication with statins, beta blockers, and angiotensin-converting enzyme inhibitors (ACE-Is)/angiotensin receptor blockers (ARBs) were selected as covariates from among the baseline variables. To assess whether potential relationships of Hcy levels and HTlase activity with all-cause death were affected by other covariates, Cox proportional hazard regression was conducted with an interaction term between Hcy levels or HTlase activity and other covariates. Thereafter, covariates with $p < 0.05$ on univariable analysis were selected for multivariable analysis. Levels of Hcy were included in the multivariate model, and hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. Whether the results differed from the cut-off points was determined using secondary analyses in which Hcy levels were treated as a continuous variable. A *p*-value <0.05 was considered significant. All data were analyzed using JMP version 11.0 for Windows (SAS Institute, Cary, NC, USA).

Results

Baseline clinical characteristics

This study included 315 consecutive patients who underwent PCI for various indications in our institution. Baseline clinical characteristics and laboratory findings of all patients and patients stratified by Hcy level are shown in Table 1. The patients' mean age was 66 ± 9 years, and 82.5% of the study population were males; 24.4% of patients were current smokers, diabetes mellitus was present in 47.6% of patients, of whom 12.4% were on insulin, while 62.5% of patients had systemic hypertension. The mean BMI was 24.0 ± 2.8 kg/m^2, and the mean total cholesterol was 178.9 ± 36 mg/dl, with a mean LDL cholesterol of 109.2 ± 31.9 mg/dl. Overall, 33% of the study population had CKD, and the mean GFR was

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