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The relationship between *S*-adenosylhomocysteine and coronary artery lesions: A case control study

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ABSTRACT

The role of homocysteine (Hcy) in the pathogenesis of coronary artery disease (CAD) is controversial, as decreased Hcy levels have not demonstrated consistent clinical benefits. Recent studies propose that *S*-adenosylhomocysteine (SAH), and not Hcy, plays a role in cardiovascular disease (CVD). We aimed to assess the relationship between plasma SAH and coronary artery lesions. Participants (n = 160; aged 40–80 years) with chest pain and suspected CAD underwent coronary angiography (CAG) for assessment of coronary artery stenosis, and were assigned to either the atherosclerosis (AS) or CAD group. Plasma SAH and *S*-adenosylmethionine (SAM) concentrations were measured and the association between coronary artery lesions and SAH was assessed. SAH levels were significantly higher in the CAD group (23.09 ± 2.4 nmol/L) than in the AS group (19.2 ± 1.5 nmol/L). While the AS group had higher values for SAM/SAH (5.1 ± 0.7 vs. 4.1 ± 1.1), levels of SAM, Hcy, folate, and vitamin B12 were similar in the two groups. Coronary artery lesions were associated with SAH (β = 11.8 [95% CI: 5.88, 17.7, P < 0.05]. Plasma SAH concentrations are independently associated with coronary artery lesions among patients undergoing coronary angiography. Plasma SAH might be a novel biomarker for the early clinical identification of CVD.

1. Introduction

Coronary artery disease (CAD) is one of the leading causes of morbidity and mortality worldwide, and the economic, social, and costs associated with treating CAD are very high [1]. The severity of coronary artery lesions has been proven to be associated with the risk of future cardiovascular events [2]. Thus, it is important to investigate new predictors of CAD to help protect against, and provide treatment for CAD. The association between homocysteine (Hcy) and vascular disease was first proposed by McCully in 1969 [3]. In addition, data from various epidemiological investigations and laboratory studies have demonstrated that an increased concentration of plasma Hcy was considered to be an independent risk factor for cardiovascular disease (CVD) [4,5]. However, large-scale clinical trials in which folic acid and vitamins B6 and B12 were supplemented to reduce the risk of major cardiovascular events in patients with vascular disease failed to show such an effect [6]. Animal research conducted by Troen et al. showed that the extent of atherosclerosis development was independent of the plasma Hcy concentration in apolipoprotein E (apoE)-deficient mice [7]. It was suggested that atherogenesis induced by a high dietary intake of methionine involves complex pathogenic mechanisms and that plasma Hcy may be an indirect indicator of coronary artery lesions.

In recent years, *S*-adenosylhomocysteine (SAH), which is the sole metabolic precursor of Hcy in a reversible reaction catalyzed by SAH hydrolase, has garnered substantial interest. Several previously conducted cross-sectional and case-control studies have indicated that plasma SAH may be a better indicator of CVD than Hcy. Kerins et al. showed that plasma SAH is a much more sensitive indicator of CVD than plasma Hcy levels [8]. Chi Liu et al. demonstrated that plasma SAH is a better biomarker of atherosclerosis than Hcy in apolipoprotein E-deficient mice that were fed high amounts of dietary methionine [9]. A prospective cohort study of 1003 patients over a 3-year period concluded that there was a positive correlation between plasma SAH levels and the risk of cardiovascular events [10]. However, the potential

Abbreviations: Hcy, homocysteine; SAH, *S*-adenosylhomocysteine; CAD, coronary artery disease; CVD, cardiovascular disease; CAG, coronary angiography; AS, atherosclerosis; SAM, *S*-adenosylmethionine; HPLC, high performance liquid chromatography

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association between plasma SAH and coronary artery lesions was not assessed. An interesting question is whether the level of SAH is a novel biomarker for the early identification of subjects at a high risk of developing CVD, before symptoms appear. Here, we performed a case control study using high-performance liquid chromatography (HPLC) to quantify the level of SAH and SAM in 162 patients, and investigated the association between plasma SAH and coronary artery lesions.

2. Materials and methods

2.1. Participants and samples

Altogether, 160 hospitalized patients with chest pain, aged between 40 and 80 years, were recruited from the General Hospital of Tianjin Medical University between March 2016 and September 2016 in Tianjin, China. Patients with AS and CAD were confirmed by coronary angiography. The inclusion criteria were stable clinical conditions, except for acute coronary syndromes, and the availability of a coronary angiogram. The exclusion criteria were the presence of liver diseases, renal insufficiency, surgery, blood diseases, hyperthyroidism, thyroid dysfunction, malnutrition, or the use of drugs, such as anticancer agents, folic acid, and vitamin B12, which would affect plasma Hcy concentrations. Patients who had undergone cardiac stent implantation were also excluded. The study was approved by the Ethical Committee of Tianjin Medical University General Hospital (Ethical No. IRB2015-YX-011), and signed informed consent was obtained from all the participants. All the protocols adhered to institutional guidelines and to the Helsinki Declaration.

Coronary angiography was performed in all the patients using the standard Judkins technique through the femoral artery or brachial artery. The angiograms were interpreted by 2 or more independent cardiologists who were unaware of the patients' risk factor profiles. All the evaluations were performed based on the protocol by the American Heart Association [11]. The CAD group was defined by $>$ 50% stenosis in at least 1 major coronary artery, including the left main coronary artery or the left anterior descending coronary artery, the right coronary, or the circumflex coronary artery. The atherosclerosis group (the AS group) was defined by $\leq$ 50% stenosis in all the major coronary arteries. Of the 160 patients, 72 patients had atherosclerosis, and the other 88 patients had coronary heart disease.

The severity of AS and CAD was expressed as the sum of the Gensini score for each lesion. Reductions in the coronary lumen diameter by 1–25%, 26–50%, 51–75%, 76–90%, 91–99%, and 100% were evaluated as a score of 1, 2, 4, 8, 16, and 32, respectively. This score was multiplied by a factor accounting for the lesion position of the coronary artery: 5 for the left main coronary artery; 2.5 for the proximal left anterior descending artery or proximal left circumflex artery; 1.5 for the mid-region of the left anterior descending artery; 1 for the distal left anterior descending artery, the mid-distal region of the left circumflex artery, or right coronary artery; and 0.5 for other segments [12].

2.2. Baseline measurement

Basic information pertaining to age, sex, smoking status (yes/no), alcohol consumption (yes/no), family history of CAD (defined as an immediate relative diagnosed with CAD before the age of 60 years), comorbid diseases (diabetes, hypertension, hyperlipidemia), and the use of medications (vitamin B12 and folate) was collected using a questionnaire during a clinical visit. The questionnaires were checked by a trained interviewer for missing data and completeness, before transferring the data to a database. During their clinical visit, participants' weight, height, and arterial blood pressure were measured using standard techniques in triplicate, and the averaged values were used for the analysis. The body mass index (BMI) was calculated by dividing the patients' weight (in kg) by their height squared (in m). Hypertension was defined as a systolic blood pressure (SBP) of 140 mm Hg or higher and/or a diastolic blood pressure of 90 mm Hg or greater or the use of antihypertensive medication. Diabetes mellitus was defined as a venous plasma glucose concentration $\geq$ 126 mg/dL after an overnight fast and/or $\geq$ 200 mg/dL 2 h after a meal or the use of insulin or oral hypoglycemic agents.

2.3. Biochemical measurements

After the patients had fasted overnight (for a minimum of 12 h), venous blood samples were collected in Ethylenediaminetetraacetic acid (EDTA) anticoagulation tubes before coronary angiography. Blood samples were immediately placed on ice and centrifuged at 3000 rpm for 10 min, then stored at − 80 °C until the start of analysis. Plasma Hcy levels were estimated by enzymatic cycling methods (Cobas 8000, Roche, Switzerland). Plasma SAH and SAM concentrations were measured using HPLC [13]. The plasma was hydrolyzed with trichloroacetic acid 400 g/L, then the hydrolysate was centrifuged and filtered using a 0.45-μm membrane. The supernatants were separated on a Venusil MP-C18 column (4.6 mm × 250 mm, 5-μm) at 30 °C with a mobile phase of 50 mmol/L NaH_2PO_4 (pH 4.38), C7 H15, NaO_3 S, and methanol, a flow rate of 1.0 mL/min, and UV detection at 254 nm. Chromatograms were recorded using an HPLC integrator, and quantification was performed by automatic peak area integration. SAM and SAH standards were used to identify the elution peaks. All samples were analyzed in triplicate.

The serum folate and serum vitamin B12 concentrations were measured using Immulite Chemiluminescent kits, based on the manufacturer's instructions and performed using IMMULITE 2000 XPi analyzer (Siemens, Germany). Levels of total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, creatinine, and fasting blood glucose were measured using routine methods. Low-density lipoprotein (LDL) cholesterol levels were measured using the colorimetric method.

2.4. Statistical analysis

The data are expressed as means and standard deviations for normally distributed continuous variables; medians and inter-quartile ranges for skewed continuous variables; and frequencies and percentages for categorical variables. Differences between the AS and CAD groups were tested using the independent student *t*-test or Wilcoxon rank-sum test for continuous variables and a chi-square test for categorical variables.

Spearman correlation analysis was used to assess associations between Gensini scores and SAH, SAM, and SAM/SAH ratio. To assess the association between coronary artery lesions and SAH, SAM, and SAM/SAH ratio, we performed multiple linear regression analyses with Gensini scores as the dependent variables, and all the potential confounders including age, sex, smoking habits, drinking habits, and BMI as independent variables. Three models were created: Model 1 did not adjust for any covariables. Model 2 adjusted for demographic characteristics, including age and sex. Model 3 adjusted for demographic characteristics and CVD risk factors, including BMI, smoking status, SBP, fasting glucose, diabetes, TC, TG, and LDL.

All statistical analyses were performed using the SPSS software (Version 21.0, SPSS, Chicago, Illinois, USA) and a two-sided P value of 0.05 was considered statistically significant.

3. Results

3.1. Baseline characteristics

Baseline characteristics of the AS and CAD groups are summarized in Table 1. The 72 patients with AS were matched to 88 CAD patients. The CAD group had a significantly higher proportion of current smokers (35.2%, $P < 0.05$) and alcohol drinkers (25%, $P < 0.05$) than the AS group. The level of HDL cholesterol in the AS group (1.3 [1.0–1.6]) was