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Levels of serum high mobility group box 1 were independently associated with cardiovascular risk in patients undergoing coronary angiography

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ABSTRACT

Background: We aimed to examine the association of serum high mobility group box 1 (HMGB1) level with cardiovascular risk in patients undergoing coronary angiography with no history of diabetes.

Methods: We enrolled patients with no history of diabetes who had been admitted for coronary angiography due to suspected or known coronary artery disease. Two to four weeks after the patients were discharged from the hospital, an oral glucose tolerance test (OGTT) was conducted and serum HMGB1 level was measured. Patients' 10-year coronary heart disease (CHD) risk was assessed using the Framingham Risk Scoring.

Results: A total of 476 patients were enrolled. Overall, mean serum HMGB1 level was 6.1 ± 1.3 pg/ml. Using linear regression analysis, high-density lipoprotein cholesterol was negatively associated with serum HMGB1 (β coefficient − 0.033, 95% CI −0.063 to −0.003, p = 0.033) after adjustment for several confounders. With regard to cardiovascular risk, levels of serum HMGB1 were positively associated with 10-year CHD risk (β coefficient 0.506, 95% CI 0.030 to 0.983, p = .037), independent of patients' undiagnosed abnormal glucose regulation.

Conclusions: In patients undergoing coronary angiography with no history of diabetes, levels of serum HMGB1 were positively associated with 10-year CHD risk, independent of patients' undiagnosed abnormal glucose regulation.

1. Introduction

High mobility group box 1 (HMGB1), a nuclear protein presented in eukaryotic cells, is a 30 kDa DNA-binding protein consisted of 215 amino acids [1–3]. The physiologic function of HMGB1 is to stabilize nucleosomes and regulate gene transcription [4–6]. In response to inflammatory stimuli, HGMB1 translocates from nuclear to cytoplasm in immune cells such as monocytes and macrophages, and is secreted outside the cells to exert a variety of biological functions [6–8]. Extracellular HMGB1 mainly binds to toll-like receptor 4 (TLR4) or the receptor for advanced glycation end product (RAGE) to activate immune or endothelial cells [9]. For example, HMGB1 increases the expression of adhesion molecules and the production of inflammatory cytokines in endothelial cells leading to vascular inflammation [10]. Hence, extracellular HMGB1 is involved in the pathogenesis of several diseases, such as sepsis, stroke, rheumatoid arthritis, and atherosclerosis [11].

With regard to atherosclerosis, Kalinina et al. [12] reported an increased expression of HMGB1 in endothelial cells, intimal smooth muscle cells, and macrophages in human atherosclerotic lesions. Moreover, Kanellakis et al. [13] reported that neutralization of HMGB1 reduces development of diet-induced atherosclerosis in Apolipoprotein

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E-deficient mice. These findings were supported by the observation that serum HMGB1 level was associated with cardiovascular diseases [14–17]. Given that serum HMGB1 level was increased in patients with newly diagnosed type 2 diabetes [18], and there was a high prevalence of undiagnosed abnormal glucose regulation in patients with cardiovascular diseases [19–22], a potential confounder in previous studies [14–16] was the fact that the glucose regulation status of patients with no history of diabetes was not confirmed with an oral glucose tolerance test (OGTT), as guidelines recommend [23,24].

Although HMGB1 has been related to cardiovascular diseases [14–17], data on the association of HMGB1 with cardiovascular risk are scarce [16,25]. In this study, we aimed to investigate the association of serum HMGB1 level with cardiovascular risk in patients undergoing coronary angiography with no history of diabetes, for whom an OGTT was conducted to determine the glucose regulation status.

2. Methods

2.1. Study design and patients

The Institutional Review Board of Taichung Veterans General Hospital, Taichung, Taiwan, approved this study which was conducted in accordance with the Declaration of Helsinki. Patients with no known history of diabetes were recruited if they were admitted for coronary angiography due to suspected or known coronary artery disease (CAD). Patients' coronary condition was confirmed according to the findings of the coronary angiography. Patients' glucose regulation status was determined based on the result of an OGTT, which was conducted after the patients were discharged from the hospital. Written informed consent was provided by all study patients prior to any study-related procedure.

2.2. Study procedures

An OGTT was conducted for all study patients two to four weeks after they were discharged from the hospital when their heart disease was deemed to be stable. Briefly, patients were fasted overnight and interviewed by a trained nurse at our outpatient clinic [21,22]. Patients' height and weight were measured, and two consecutive readings of blood pressure from the upper arm were recorded with a 30-second interval between readings. The average of the two blood pressure readings was used for analysis. Before a standard 75-g OGTT [26] was conducted, a blood sample was obtained for fasting plasma glucose, insulin, HMGB1, and lipids measurements. An OGTT was then performed and a blood sample was collected at 120 min for the measurement of plasma glucose.

2.3. Biochemical analyses

Levels of serum HMGB1 were determined using an ELISA kit (HMGB1 ELISA kit, Catalog No. LS-F4038; LifeSpan BioSciences, Inc. Seattle, WA, USA) following the manufacturer's instructions. The minimal detectable concentration of HMGB1 was 1.56 ng/ml, and the intra- and inter-assay coefficients of variation were < 10% and < 12%, respectively. An ELISA kit (Human CRP Quantikine ELISA kit, R&D Systems) was used to determine levels of serum high-sensitivity C-reactive protein (hsCRP). The intra- and inter-assay coefficients of variation were both < 8.3%, and the minimum detectable concentration of hsCRP was 0.001 μg/dl. Levels of plasma glucose were measured using the glucose oxidase-peroxidase method (Wako Diagnostics, Tokyo, Japan). The intra- and inter-assay coefficients of variation for plasma glucose were both < 1.5%. Levels of plasma insulin were determined using electrochemiluminescence immunoassay (Elecsys 2010; Roche Diagnostics, Indianapolis, IN). The respective coefficient of variation for intra- and inter-assay of plasma insulin was 1.8% and 2.5%. Levels of serum lipids were determined using UniCel DxC Systems (Beckman Coulter, Inc.). The respective coefficient of variation for intra- and inter-assay of serum lipids was < 3.0% and < 4.5%.

2.4. Definition of study objectives

Patients' fasting and 2-hour plasma glucose in the 75-g OGTT were used to determine their glucose regulation status, as recommended by the American Diabetes Association [27]. Insulin resistance and β-cell function were calculated using the homeostasis model assessment (HOMA) method (represented as HOMA-IR and HOMA-β, respectively) [28]. CAD was defined as ≥50% stenosis of the lumen diameter in any coronary artery as demonstrated by the coronary angiography. Patients' 10-year coronary heart disease (CHD) risk was assessed using the Framingham Risk Scoring [29].

2.5. Statistical analysis

All of the statistical analyses were performed using the Statistical Package for the Social Science (IBM SPSS version 22.0; International Business Machines Corp, NY, USA). Continuous variables are reported as mean ± SD, while categorical data are given as numbers (percentages). Differences in levels of serum HMGB1 between subgroups of study patients were tested for statistical significance with the independent *t*-test or ANOVA. Univariable and multivariable regression analyses were used to examine the association of HMGB1 with clinical factors and estimated 10-year CHD risk. The optimal cut-off value of HMGB1 to identify a 10-year CHD risk ≥10% was determined using receiver operating characteristic (ROC) analysis. In all statistical analyses, a two-sided P value < 0.05 was considered statistically significant.

3. Results

Table 1 shows the baseline characteristics of study patients. A total of 476 patients were included in the analysis (mean age 61 ± 12 years, male 81.9%, mean body mass index 26.1 ± 3.7 kg/m^2), among whom 275 (57.8%) had coronary angiography-proven coronary artery disease. With regard to glucose regulation status, the proportion of patients with normal glucose tolerance, prediabetes, or newly diagnosed diabetes was 41.0, 43.7, and 15.3%, respectively, based on the results of OGTT.

Table 1
Characteristics of all study patients.

N	476
Age (years)	61 ± 12
Male, n (%)	390 (81.9)
Body mass index (kg/m^2)	26.1 ± 3.7
Smoking, n (%)	167 (35.1)
CAD proved by coronary angiography, n (%)	275 (57.8)
Systolic blood pressure (mm Hg)	127 ± 18
Diastolic blood pressure (mm Hg)	74 ± 11
Total cholesterol (mmol/L)	4.4 ± 1.0
Low-density lipoprotein cholesterol (mmol/L)	2.5 ± 0.8
High-density lipoprotein cholesterol (mmol/L)	1.2 ± 0.3
Triglycerides (mmol/L)	1.5 ± 0.9
Oral glucose tolerance test	
Fasting plasma glucose (mmol/L)	5.3 ± 0.7
2 h plasma glucose (mmol/L)	8.3 ± 2.7
Glucose regulation status, n (%)	
Normal glucose tolerance	195 (41.0)
Prediabetes	208 (43.7)
Newly diagnosed diabetes	73 (15.3)
HOMA-IR	2.7 ± 3.6
HOMA-β	129 ± 100
High sensitivity C-reactive protein (μg/ml)	2.2 ± 2.1
Framingham 10-year CHD risk (%)	11.5 ± 8.1

Values are mean ± SD or n (%). CAD, coronary artery disease. HOMA, homeostasis model assessment. IR, insulin resistance. CHD, coronary heart disease.