Plasma Levels of C1q/TNF-Related Protein 1 and Interleukin 6 in Patients With Acute Coronary Syndrome or Stable Angina Pectoris

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Abstract: *Background:* C1q/TNF-related protein 1 (CTRP-1), a novel adipocyte factor, may participate in the mechanisms of metabolism and inflammation. Interleukin 6 (IL-6) is a proinflammatory cytokine that is correlated with the severity of coronary heart disease (CHD). In this study, we focused on the levels of CTRP-1 and IL-6 in patients with CHD. *Methods:* Circulating CTRP-1 and IL-6 levels were measured using enzyme-linked immunosorbent assay in 81 patients with acute coronary syndrome (n = 41) or stable angina pectoris (n = 40). CTRP-1 and IL-6 levels were also examined in 30 healthy individuals (control group). We examined the correlations between the levels of CTRP-1 and IL-6 and cardiac risk factors in CHD. Logistic regression analysis was performed to screen for factors that predict CHD. *Results:* Both CTRP-1 and IL-6 concentrations were increased in the acute coronary syndrome or stable angina pectoris group compared with the control group ($P < 0.01$). Both plasma levels of CTRP-1 and IL-6 in the single-, double- and triple-vessel lesion group were higher compared with the control group ($P < 0.01$). CTRP-1 levels were positively correlated with IL-6 ($r = 0.667$, $P < 0.01$) and high-sensitivity C-reactive protein levels ($r = 0.520$, $P < 0.01$) and negatively correlated with HDL-C ($r = -0.432$, $P < 0.01$). The logistic regression analysis showed that increases in CTRP-1 and IL-6 levels may be powerful predictors of CHD. *Conclusions:* The variation of plasma CTRP-1 and IL-6 concentrations may play an important role in reflecting the degree of inflammation in CHD and the severity of coronary arterial atherosclerosis. This potential suggests that evaluating CTRP-1 and IL-6 in combination may aid in predicting the occurrence of CHD.

Key Indexing Terms: C1q/TNF-related protein 1; Interleukin 6; Coronary heart disease; Acute coronary syndrome; Stable angina pectoris.
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Coronary heart disease (CHD) is one of the major cardiovascular diseases, which has seriously impacted human health globally. CHD is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial tree.[1]

As a vital endocrine organ, adipose tissue secretes various types of bioactive substances, known as adipokines, that play important roles in the pathogenesis of obesity, diabetes, hypertension and cardiovascular diseases.[2] Recently, a new protein family secreted by adipokines, C1q/TNF-related protein (CTRP), was cloned on the basis of its sequence homology with adiponectin.[3] This family of adiponectin paralogs may be involved in energy homeostasis and obesity-related inflammation.[4]

C1q/TNF-related protein 1 (CTRP-1), a member of the CTRP family, is secreted by stromal vascular cells (SVCs) composed of adipose-tissue macrophages, preadipocytes and endothelial cells.[5] In human tissues, CTRP-1 mRNA is expressed more highly in the heart than in many other tissues such as the liver and kidney.[6] Increased CTRP-1 gene expression can be indirectly induced by lipopolysaccharide (LPS) in epididymal adipose tissue and is regulated by the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in association with a decrease in adiponectin mRNA expression,[7] indicating that adipose tissue CTRP-1 expression is induced in inflammation. In addition, Lasser et al[8] demonstrated that CTRP-1 could specifically bind to fibrillar collagen type I and block collagen-induced platelet activation and aggregation. These observations indicate that CTRP-1 may play a role in the process of plaque formation in CHD.

Interleukin 6 (IL-6) is a proinflammatory cytokine that is triggered by vulnerable plaque or necrotic myocardium and is correlated with the severity of coronary artery disease (CAD).[9] In patients with CAD, elevated circulating IL-6 concentrations may be involved in the function of macrophage/foam cells present in atheromatous plaques.[10]

At present, data are limited regarding the correlation between plasma concentrations of CTRP-1 and IL-6 and the severity of CHD. Therefore, in this study, we measured plasma concentrations of these biomarkers and investigated their relationship with disease severity in patients with CHD.

METHODS

Study Participants and Definition of Coronary Heart Disease

The study was approved by the Institution Review Board of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from every participant.

From May 1, 2013, to February 1, 2014, 81 consecutive patients with CHD (51 men; mean age: 56.48 ± 10.02 years; range, 39–85) were recruited from the Department of Cardiology in the First Affiliated Hospital of Zhengzhou University. All patients had CHD confirmed by coronary angiography (CAG). The enrolled patients were classified into 2 groups: a group with acute coronary syndrome (ACS) and a group with stable angina pectoris (SAP). The ACS group consisted of 20 patients with ST-elevation acute myocardial infarction and 21 patients with unstable angina pectoris (UAP). The SAP group consisted of 40 patients with SAP. Percutaneous coronary

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intervention was performed in 81 patients with ACS and SAP. During the same period, 30 healthy subjects (18 men; mean age: 50.87 ± 10.56 years; range, 34–77) were recruited as a control group, excluding individuals with a history of CVD (myocardial infarction, unstable angina, stroke or cardiovascular revascularization), type 2 diabetes, stage 2 hypertension (resting blood pressure ≥160/100 mm Hg), malignancy or severe renal or hepatic disease.

Acute myocardial infarction (AMI) was defined as a typical increase and gradual decrease of biochemical markers of myocardial necrosis (detection of a rise and/or fall of cardiac biomarkers such as CK-MB and/or troponin-T with at least 1 value above the 99th percentile upper reference limit) and at least 1 of the following:

Ischemic symptoms, electrocardiogram changes indicative of new ischemia (new ST-T changes or new left bundle branch block), development of pathologic Q waves on electrocardiogram and imaging evidence of a new loss of viable myocardium or new regional wall motion abnormality.[11] The diagnostic criteria for UAP included chest pain at rest with either a ST segment depression of at least 0.1 mV or a T-wave inversion in 2 or more continuous electrocardiographic leads and no biomarkers of myocardial necrosis (based on 2 or more blood samples collected at least 6 hours apart, with a reference limit of the 99th percentile of the normal population).[12] In case of SAP, the criteria included chest pain for at least 6 months accompanied by evidence of severe CAD on CAG, with no clinically evident ischemic episodes during the week preceding arteriography.[13]

Exclusion criteria were a history of cardiomyopathy, valvular heart disease, severe renal failure (creatinine >2.5% mg), respiratory insufficiency, severe hepatic disease, malignant neoplasia or terminal cachexia, active infective diseases, peripheral angiopathy, cerebral vessel diseases and mental disorders or linguistic barriers that impeded adequate comprehension and collaboration.

Data Collection

Trained research assistants, who retrospectively reviewed all individual medical records, undertook the data collection. All data collection was conducted with quality control. Demographic characteristics (age and sex) and data regarding the presence of coronary risk factors (body mass index [BMI], hypertension, type 2 diabetes, hyperlipidemia, family history of CHD, smoking status and drinking history) were collected. In addition, laboratory measurements were conducted by the clinical laboratory of the First Affiliated Hospital of Zhengzhou University, including CK-MB, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), high-sensitivity C-reactive protein (hs-CRP) and N-terminal probrain natriuretic peptide. The left-ventricular ejection fraction (%) was calculated by 2-dimensional echocardiography using a digital imaging system (Vivid-7; GE Medical System, Willoughby, OH).

Measurements of CTRP-1 and IL-6

Blood samples used for the assessment of plasma CTRP-1 and IL-6 levels were obtained from all individuals by venipuncture after admission to the hospital and 15 minutes of bed rest and collected in tubes containing EDTA and aprotinin. Plasma samples were separated by centrifugation at 4°C at 3,000 rpm for 10 minutes, then subsequently frozen and stored at −80°C until assayed in a blinded manner in a single batch. The levels of expressed CTRP-1 were detected using enzyme-linked immunosorbent assay kits (Elabscience Biotechnology Co Ltd, Wuhan, China), and IL-6 levels were measured by enzyme-linked immunosorbent assay (Nanjing Jiancheng Technology Industry Co Ltd, Nanjing, China). These assay kits had a lower limit of detection of 0.19 ng/L for CTRP-1 and 2.00 ng/L for IL-6. We performed every process of the experiment with strict quality control. And the quality control procedure for all blood samples and the detection of CTRP-1 and IL-6 were completed. More than 90% of our samples exhibited a normal odds ratio.

Coronary Angiography

CAG was performed using standard Judkins' techniques. Two experienced interventional cardiologists who were unaware of the subjects' clinical information performed the angiographic analysis. CHD was defined as the presence of a 50% or greater decrease in internal diameter in at least 1 main coronary artery. The Gensini's score and the number of involved coronary branches were used to assess the extent and severity of CAD. According to the results of CAG, patients with CHD were further grouped according to the number of significantly stenotic vessels, defined as a decrease in internal diameter of more than 50%. These patients were grouped into single-vessel, double-vessel and triple-vessel disease groups.

Statistical Analysis

The data were analyzed with SPSS version 20.0 (IBM SPSS Inc, Chicago, IL). Continuous variables were presented as the mean ± SD or median (interquartile range). The Shapiro-Wilk's test was performed to evaluate normality. Categorical variables were presented as absolute and relative frequencies. Mean values in groups were compared using parametric statistics (Student's *t* test and analysis of variance) or nonparametric statistics (Mann-Whitney and Kruskal-Wallis' tests), depending on the distribution of the variable of interest. Fisher's exact test was applied when comparing the number of patients with type 2 diabetes mellitus or hyperlipidemia in the ACS, SAP and control groups. Bonferroni's correction was used to adjust for multiple comparisons. If distributions of both variables were normal, we selected the Pearson's test for correlation analysis; and if the distributions of variables were not normal, the Spearman's test was chosen. The univariate logistic regression analysis was first used to screen the significant independent variables and then followed by the multivariate logistic regression analysis with screened categorical variables and with or without CHD as dependent variables using the forward stepwise (conditional) method to identify the risk factors. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Characteristics of Subjects

Comparisons of the clinical and biochemical characteristics of the study subjects are shown in the Table 1. Among the 3 groups, there was no significant difference in gender, BMI, medical history of hypertension, family history of CAD, drinking history, heart rate, systolic blood pressure (SBP), TC, TG or LDL-C. A greater number of patients with ACS had a history of smoking compared with those in the SAP group ($P < 0.01$); in addition, subjects with ACS had significantly lower levels of HDL-C compared with the SAP and control groups ($P < 0.01$). Subjects with SAP were significantly older compared with the control group ($P < 0.05$), and the ACS group had a higher diastolic blood pressure (DBP) compared with the SAP group ($P < 0.05$). Much more patients had a history of type 2 diabetes mellitus and hyperlipidemia in the SAP group compared with the control group ($P < 0.01$), moreover the number of patients