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Original article

Plasma homocysteine is a determinant of tissue necrosis factor-α in hypertensive patients

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Abstract

Chronic sub-clinical inflammation observed in hypertension plays a prominent role in the progression of atherosclerosis. Accumulating evidence suggests that homocysteine (Hcy) can cause inflammation. The aim of this study was to compare the predictive utility of Hcy and lipid measures as determinants of inflammation in hypertensive patients. We studied a group of 100 patients (45.0 ± 12.2 years old) with essential hypertension and a control group of 40 healthy volunteers (44.0 ± 8.7 years old). We found that plasma total Hcy (tHcy), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and C-reactive protein (CRP) were significantly higher in hypertensive patients compared with the control group. The subgroup of hypertensive patients with obesity had higher levels of TNF-α ($p < 0.001$), IL-6 ($p < 0.01$), and tHcy ($p = 0.063$), compared with the subgroup of hypertensive patients without obesity. The subgroup of patients with a history of myocardial infarction or stroke had significantly higher levels of tHcy, TNF-α, IL-6, and CRP compared to patients with a negative history of vascular events. In the group of hypertensive patients, a strong positive correlation between tHcy and TNF-α was observed ($r = 0.48$; $p < 0.001$). In contrast, no correlation was observed between TNF-α and any of the lipid measures. In multivariate regression analysis tHcy, but not lipids, was an independent predictor of TNF-α. In conclusion, our findings show that plasma tHcy is a determinant of TNF-α in hypertension and that obesity or a history of vascular events aggravates inflammation in patients with hypertension. A positive correlation between Hcy and TNF-α suggests a mechanism underlying the pro-atherogenic properties of Hcy.

Keywords: Homocysteine; Hypertension; TNF-α

1. Introduction

Since 1969, when McCully reported extensive atherosclerotic changes in children with elevated plasma total homocysteine (tHcy) caused by inborn errors in methionine metabolism [1], numerous clinical studies have shown that hyperhomocysteinemia is an independent risk factor for coronary, cerebral, peripheral artery disease, and venous thrombosis [2]. The importance of hyperhomocysteinemia in the pathogenesis of atherosclerosis is highlighted by the fact that 50% of all patients with genetic homocystinuria show atherosclerotic changes before 30 years of age [1,2].

The molecular mechanism by which hyperhomocysteinemia exerts its atherogenic effect is a subject of intense studies. Hcy excess leads to endothelial dysfunction, decreased bioavailability of nitric oxide, oxidative stress, protein modification and endoplasmic reticulum stress [3,4], and thrombosis [5]. The accumulation of Hcy can also induce pro-inflammatory reactions

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[6–8]. For example, hyperhomocysteinemia is associated with markers of immune activation, such as neopterin [7] or autoantibodies against *N*-Hcy-protein, which are elevated in human stroke and CAD patients [8].

Over the past years the role of innate immunity and inflammation in the development of cardiovascular disease has gained much attention [9]. Arterial hypertension – a classical risk factor for atherosclerosis – through persistently elevated blood pressure leads to mechanical damage of the endothelium. Abnormal shear stress results in the dysfunction of endothelial cell and induces low-grade inflammatory state [10]. In obesity, adipocytes can function as endocrine cells: they do not only serve as a regular fat storage but also secrete a variety of bioactive substances known as adipocytokines [*e.g.*, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α)], which creates an inflammatory environment [11]. Furthermore, accumulating evidence suggests that obesity influences acute phase proteins' (APPs) production [12]. APPs form a homogenous group of serum proteins whose role is to restore disturbed homeostatic balance in the body. Much interest has been devoted to the elevated serum concentration of C-reactive protein (CRP) – a marker of strong predictive power for cardiovascular events – in obese subjects [13].

However, determinants of inflammation in patients with hypertension are not well understood. The aims of the present study are to test a hypothesis that Hcy is a predictor of inflammation and to determine relative contributions of Hcy and lipids to inflammation in patients with hypertension.

2. Methods

2.1. Participants

We studied 100 patients (43 men and 57 women, mean age 45.0 ± 12.2) with essential hypertension (I stage hypertension according to JNC VII) housed in a hospital ward and 40 healthy controls (11 men and 29 women, mean age 44.0 ± 8.7). The study was approved by the Human Subjects Oversight Committee, University of Medical Sciences, Poznan and all participants provided informed consent.

2.2. Study protocol and definitions

All participants underwent routine physical examination. Resting seated blood pressure was measured three times and an average value was calculated according to The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC VII) guidelines. Anthropometric measurements were performed; body mass index (BMI) was calculated using the standard formula. Obesity was defined as BMI ≥ 30 kg/m^2. Based on a patient's history, physical examination and basic laboratory analysis any clinically evident acute and/or chronic inflammatory process (within the respiratory, digestive and genitourinary tract, oral cavity, pharynx and paranasal sinuses) was excluded. Patients from both the study and control groups were asked to restrain from smoking for the time of the study. Percentage of smokers was similar in both groups. Percentage of hypertensive patients using medications was as follows: statins, 31%; aspirin, 26%; ACE inhibitors, 36%; calcium-channel blockers, 23%; diuretics, 26%, beta blockers, 19%.

2.3. Laboratory measurements

Blood samples were taken after an overnight fast and after 30 min in the supine position. Plasma tHcy was determined by immunoassay using IMX analyzer (Abbott Laboratories). TNF-α concentration was assayed by IRMA with reagents from Bisource Europa Ltd, IL-6 – by ELISA with reagents from R&D System Europa Ltd. CRP was assayed according to Laurell rocket immunoelectrophoresis method. Plasma total cholesterol (TCH), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatinine and glucose were measured using commercial kits.

2.4. Statistical analysis

Data are shown as mean ± SD. All calculations and statistics were performed with Statistica for Windows (version 5 '97 edition). The differences between the groups were tested by one-way analysis of variance. In case the P value was <0.05, the groups were compared by the appropriate test (Student's test for unpaired samples). Simple associations between variables will be calculated as the Pearson coefficient of correlation. Two explorative multiple-regression models, stepwise regression, and all possible subsets regression were used to identify the combination of variables with the best predictive value [14]. The following variables or transformations of these were tested both in simple-regression analyses and in the multiple-regression models: age, gender, smoking status, history of myocardial infarction or stroke, BMI, SBP, DBP, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, fasting glucose, creatinine, TNF-α, IL-6, CRP and tHcy. A P value of <0.05 was regarded as significant. Logarithmic transformation was used to normalize non-normally distributed dependent variables.

3. Results

Characteristics of the hypertensive and control groups are shown in Table 1. Hypertensive subjects had significantly higher plasma tHcy, TNF-α, IL-6, and CRP than healthy subjects (Table 2). In the subgroup of hypertensive patients with obesity ($n = 50$) significantly higher levels of TNF-α and IL-6 were observed when compared to hypertensives without obesity ($n = 50$) (Table 3). Higher plasma tHcy was also observed in the subgroup of obese hypertensive patients compared to hypertensives without obesity, but the difference did not reach statistical significance ($P = 0.063$).

Patients with a history of myocardial infarction or stroke ($n = 18$) had significantly higher levels of tHcy, TNF-α, IL-6 and CRP, compared to patients with negative history ($n = 82$) (Table 4). A significant positive correlation between plasma tHcy and TNF-α was observed ($r = 0.48$; $p < 0.001$)