ELSEVIER

Atherosclerosis 182 (2005) 121–127

ATHEROSCLEROSIS

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Correlation of plasma homocysteine level with arterial stiffness and pulse pressure in hemodialysis patients

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Received 17 July 2004; received in revised form 9 January 2005; accepted 28 January 2005
Available online 25 February 2005

Abstract

Elevated plasma homocysteine, arterial stiffness, and increased pulse pressure (PP) are independently associated with higher cardiovascular risk in patients with end-stage renal disease. The aim of this study is to investigate the influence of plasma homocysteine on arterial stiffness and PP in hemodialysis (HD) patients. One hundred and nine HD patients were stratified into three groups by plasma homocysteine levels: low (11.2–20.8 μmol/L), middle (21.2–25.1 μmol/L), and high tertiles of plasma homocysteine (Hcy) group (25.2–43.9 μmol/L). Using a computerized oscillometry, we measured the arterial stiffness index (ASI) and blood pressure (BP) hemodynamic parameters in the brachial artery. The high Hcy group exhibited a higher ASI (110.4 ± 129.5 versus 46.2 ± 17.5, mean ± S.E., $P < 0.01$), PP (59.7 ± 23.1 versus 43.3 ± 16.3 mmHg, $P < 0.01$), and age (57.8 ± 14.1 versus 49.9 ± 12.7 years, $P < 0.05$) compared with the low Hcy group. Plasma homocysteine was significantly correlated with ASI ($r = 0.25$, $P < 0.001$), PP ($r = 0.33$, $P < 0.001$), systolic BP ($r = 0.31$, $P < 0.001$), and age ($r = 0.24$, $P < 0.05$). Serum ferritin was significantly correlated with ASI ($r = 0.24$, $P < 0.05$) and PP ($r = 0.23$, $P < 0.05$). ASI was also correlated with PP ($r = 0.64$, $P < 0.001$). Multiple regression analyses showed that both plasma homocysteine and serum ferritin had significant associations with ASI ($\beta = 4.246$, $P = 0.007$ and $\beta = 0.024$, $P = 0.006$, respectively), and with PP ($\beta = 1.089$, $P = 0.002$ and $\beta = 0.005$, $P = 0.005$, respectively) independent of other classic risk factors for atherosclerosis. In conclusion, plasma homocysteine, along with serum ferritin, may act as an important predictor for arterial stiffness and PP in HD patients.

Keywords: Homocysteine; Arterial stiffness; Pulse pressure; Ferritin; Hemodialysis; End-stage renal failure

1. Introduction

Cardiovascular disease is the leading cause of mortality among patients with end-stage renal disease (ESRD) [1]. Elevated plasma homocysteine levels have been established as an independent risk factor for atherosclerotic cardiovascular disease in patients with ESRD [2,3]. Moreover, arterial stiffness has been associated with higher cardiovascular events and mortality in general population and in patients with ESRD [4,5]. Arterial stiffness is modulated by many risk factors related to atherosclerosis, such as age, diabetes mellitus (DM), arterial calcification, hypertension, smoking, dyslipidemia, and renal failure [6].

Previous studies have reported the association of plasma homocysteine with arterial stiffness [7–11]. However, those results remain controversial because of the differences in study population and in the methods of assessing arterial stiffness. Acute raising of plasma homocysteine concentration through methionine loading induced arterial stiffness

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doi:10.1016/j.atherosclerosis.2005.01.038

in healthy subjects in the study by Nestel et al. [7], but not in the study by Wilkinson et al. [8]. Moreover, plasma homocysteine levels were independently correlated with arterial stiffness measured as aortic pulse wave velocity (PWV) in hypertensive patients [9], and in lower-limb PWV in patients with ESRD [10]. Nevertheless, reducing plasma homocysteine levels by folic acid did not change the carotid artery stiffness in patients with ESRD [11]. Therefore, it needs further investigation to clarify the relationship of plasma homocysteine with arterial stiffness in patients with ESRD.

Moreover, increased PP has been associated with higher cardiovascular mortality in patients with ESRD [12,13]. Determination of PP depends on cardiac ejection and the properties of arterial wall [14]. Stiffness of the aorta and large arteries may be principally responsible for increased PP. Previous studies have demonstrated that plasma homocysteine might regulate the blood pressure (BP) and PP, although the consensus has not been reached [15–18]. Thus, the link of plasma homocysteine with arterial stiffness and PP in patients with ESRD remains to be determined.

To assess arterial stiffness, many noninvasive methods have been developed and they usually require expertise techniques [19]. Arterial stiffness index (ASI) is a quantitative marker for arterial stiffness by measuring the volumetric changes of brachial artery in a computerized oscillometry device [20]. ASI was proved to be significantly correlated with aortic stiffness measured by PWV, and could serve as a reliable and convenient method for arterial stiffness [21,22]. Accordingly, the aim of this study is to investigate the influence of plasma homocysteine and other risk factors for atherosclerosis on the ASI and PP in patients with ESRD on maintenance hemodialysis (HD).

2. Subjects and methods

2.1. Study population

The study population consisted of 109 stable HD patients (49 males and 60 females, with a mean ± S.D. age 53 ± 13 years) with the duration for more than 3 months. HD procedures were carried out by a 4-h session three times weekly using low flux hollow fiber dialyzers and bicarbonate dialysates containing calcium concentrations from 2.5 to 3.0 mEq/L. Dialysis efficacy was evaluated as *KT*/*V* using the equation of Gotch [23]. Patients with malignancies, pregnancy, and major infection were excluded. Folic acid or vitamin B12 was not prescribed at least three months before the study. Patients were stratified into three groups: low (11.2–20.8 μmol/L), middle (21.2–25.1 μmol/L), and high (25.2–43.9 μmol/L) tertiles of plasma homocysteine levels. Informed consent was obtained from each participant in this study that was approved by our institutional review board.

2.2. Arterial stiffness index (ASI) of brachial artery

The ASI of brachial artery was determined by a computerized oscillometry device (CardioVision, MS-200 model, International Medical Devices, Las Vegas, NV, USA). This device simultaneously measured the stiffness index of brachial artery, systolic and diastolic BP, and pulse pressure. The procedure and principle were described briefly as follows according to the instruction's manual: ASI and BP hemodynamic parameters were measured by a sensor in the cuff wrapped around the brachial artery in the upper arm without arteriovenous fistula. The pulsation and volumetric changes of the brachial artery were recorded as the cuff pressure was steadily declined. ASI was calculated as the pressure width (mmHg ×10) of oscillometric curve at 80% of mean arterial pressure. Therefore, arteries with lower elasticity were presented with the rounded oscillometric curve and the higher ASI values. We performed the tests three times in each patient to obtain the average. To evaluate the reproducibility of measurements, we obtained the coefficients of variation of 7.9% on repetitive measurements for five times, and 13.1% on day-to-day measurements from five HD patients.

2.3. Biochemical assays

Blood samples were centrifuged within 1 h of collection and were frozen at −20 °C until analysis Plasma homocysteine levels were determined by fluorescence polarization immunoassay using an IMx® Homocysteine kit (Abbott Laboratories, Abbott Park, IL, USA). Blood levels of folic acid and vitamin B12 were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Biochemical parameters were measured by an autoanalyzer (Hitachi 736-40, Tokyo, Japan).

2.4. Analysis of MTHFR C667T genotypes

The 667 C to T transition in the MTHFR gene was identified by the method of Frosst et al. [24]. In brief, DNA was extracted from leukocytes and amplified by PCR using the following forward and reverse primers: 5′-AGGACGTGAAGGAGAAGGTGTCTGCGGGA-3′ and 5′-AGGACGGTGCGGTGAGAGTG-3′. Thermal cycling conditions consisted of 40 cycles of denaturation at 95 °C for one minute, annealing at 60 °C for one minute, and extension at 72 °C for one minute. The 198 bp PCR product was digested by *Hinf*1 restriction enzyme to identify C667T mutation. MTHFR genotypes were indicated by the uncut 198 bp fragment in wild type, three fragments (198 bp, 175 bp and 23 bp) in the heterozygous mutation (CT), and two fragments (175 bp and 23 bp) in homozygous mutation (TT).

2.5. Statistical analysis

Data are expressed as mean ± S.D. Unpaired *t*-test and one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison tests were used to compare