Association Between Serum Homocysteine, Vitamin B12, Folate and Thai Coronary Artery Disease Patients

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Abstract

Background: Homocysteine is an intermediate compound formed during metabolism of methionine. The plasma level of homocysteine is dependent on the genetically regulated level of essential enzymes and the intake of folic acid, vitamin B6 (pyridoxine), and vitamin B12 (cobalamine). Elevated serum homocysteine levels are a known risk factor for coronary artery disease (CAD). To establish the magnitude of the CAD that is associated with an increased serum homocysteine level, we compared CAD patients with normal healthy Thai controls.

Method: In a cross-sectional study design we investigated the association between serum homocysteine, vitamin B12 and folate levels and the coronary heart disease in 178 CAD patients and 178 normal healthy controls by age and sex matching. These comprised 266 men and 90 women, mean age 58 $\pm$ 10 years for normal controls and 60 $\pm$ 10 years for CAD patients. Serum homocysteine, vitamin B12 and folate were measured by ELISA method and electrochemiluminescense method respectively.

Results: Paired t-test analysis showed that serum homocysteine concentrations were significantly higher in CAD patients (23.83$\pm$11.29 μmol/L) than in control subjects (19.69$\pm$8.51 μmol/L; $p < 0.001$). Homocysteine levels were also higher in males than in females. These findings were similar in healthy controls (male: 20.37 $\pm$ 8.5 μmol/L, female: 17.77 $\pm$ 8.2 μmol/L, $p < 0.05$) and in CAD patients (male: 24.91 $\pm$ 11.8 μmol/L, female: 20.73 $\pm$ 8.9 μmol/L, $p < 0.05$). Homocysteine above 17 μmol/L occurred more common in CAD patients than in control groups (OR = 1.65, 95%CI = 1.09-2.52, $p = 0.0249$). Low levels of vitamin B12 and folate did not reaching statistical significance when comparing controls and CAD patients.

Conclusions: Serum homocysteine concentrations were significantly higher in CAD patients than in controls. Serum vitamin B12 and serum folate levels were not statistically significantly different between CAD patients and control groups. The data also demonstrated that the serum homocysteine level is almost always higher in men than in women as previously reported. Although serum vitamin B12 and serum folate levels were not below the upper limit

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of normal, vitamin B12 and folic acid treatment may reduce serum homocysteine concentrations in CAD patients. We hope that the reversible risk factors will be concern to clinicians for the reduction in the risk of myocardial infarction.

Key word : Homocysteine, B12, Folate, CAD Patients

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Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. Homocysteine is metabolized by two pathways: the remethylation pathway which needs folate and vitamin B12 in these reactions and the transulfuration pathway which requires vitamin B6 as a catalyst[1]. The elevation of total circulating homocysteine concentrations in a fasting state is associated with an increased risk of occlusive vascular disease and increased mortality in individuals with previously diagnosed vascular disease. This relation appears to be an independent risk factor[2-4]. Several factors have been demonstrated with elevated tHcy concentration in humans. These factors have been attributed to heterozygosity for cystathione ß-synthase, a thermolabile variant of the methylenetetrahydrofolate reductase and dietary factors, especially vitamin B12, B6 and folate. Previous studies have demonstrated that elevated plasma tHcy levels, low plasma folate and low plasma vitamin B12 levels were associated with an increased risk of coronary artery disease (CAD)[5,6].

The aim of the present study was to investigate the relationship between hyperhomocysteinemia, serum vitamin B12, serum folate levels in CAD patients. We also attempted to determine the prevalence and the relative risk of mild hyperhomocysteinemia in CAD patients when compared with normal control subjects.

MATERIAL AND METHOD

The present study is a cross-sectional study conducted between 1997 and 1998. We investigated the association between serum homocysteine, vitamin B12 and folate levels with ischemic heart disease in 178 CAD patients and 178 normal healthy controls by age and sex matching. These comprised 266 men and 90 women, mean age 58 ± 10 years for normal controls and 60 ± 10 years for CAD patients. CAD was diagnosed if the patients had a documented clinical history of myocardial infarction or electrocardiographic evidence of Q-wave myocardial infarction (n = 5) or typical angina pectoris without previous myocardial infarction (n = 173). The control group was enlisted from healthy volunteers and from out patient departments who attended the general clinics with nonrelated problems. The controls were selected so that they were age and sex-matched to the patients. They were routinely questioned about symptoms of CAD. Those who had symptoms or signs of CAD and those who had renal impairment (creatinine > 2.5 mg/dL) were excluded from the study.

Homocysteine assay

Clotted blood was collected by venepuncture from the patients and controls and immediately centrifuged at 3,000 g for 15 minutes. The serum was separated within 1 hour after venepuncture and was stored at –70°C until analysis. Homocysteine in serum was determined by using Axis homocysteine EIA (BioRad, USA) as previously described[7,8]. In brief, protein-bound homocysteine is reduced to free homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) by the use of SAH hydrolyze and excess adenosine. The solid-phase enzyme immunoassay is based on competition between SAH in the sample

and immobilized SAH bound to the walls of the microtiter plate for binding sites on a monoclonal anti-SAH antibody. A secondary rabbit anti-mouse antibody labeled with the enzyme horseradish peroxidase (HRP) is added. The peroxidase activity is measured spectrophotometrically after addition of substrate. The absorbance is inversely related to the concentration of total homocysteine in the sample.

Serum vitamin B12 and folate concentrations were determined by electrochemiluminescence immunoassay, Elecsys 2010 (Roche, Switzerland).

Statistical method

StatView (Abacus, USA) and Epiinfo (CDC, USA) statistical programs were used for analysis. Values of $p < 0.05$ were considered statistically significant. Use of ANOVA for normal distribution, the Kruskal-Wallis test for variables with a skewed distribution, and overall χ^2 test for categorical variables evaluated differences in these baseline characteristics.

RESULTS

Total serum homocysteine concentrations were significantly higher in the CAD patients (23.83 ± 11.29 μmol/L) than in the control subjects (19.69 ± 8.51 μmol/L; $p < 0.001$, Table 1). Table 2 shows the comparison of serum homocysteine above 17 μmol/L, serum vitamin B12 below 200 ng/L and serum folate below 3 μg/L in controls and CAD patients. We found that homocysteine above 17 μmol/L occurred more commonly in CAD patients than in the control group (OR = 1.65, 95%CI = 1.09-2.52, $p = 0.0249$). Low levels of vitamin B12 and folate did not reach statistical significance when compared between controls and CAD patients. Homocysteine levels were also higher in males than in females. These findings were seen in both controls (male: 20.37 ± 8.5 μmol/L, female: 17.77 ± 8.2 μmol/L, $p < 0.05$) and CAD patients (male: 24.91 ± 11.8 μmol/L, female: 20.73 ± 8.9 μmol/L, $p < 0.05$, Fig. 1). Vitamin B12 levels were not statistically different between males and females in

Table 1. Demographic, biochemical parameters, homocysteine, vitamin B12, and folate levels of 178 CAD patients and 178 controls.

value	Controls (n = 178)	CAD patients (n = 178)	p
Age (year)	58 ± 10	60 ± 10	NS
Sex, Male/Total (%)	133/178 (75)	133/178 (75)	NS
Cholesterol (mg/dL)	216 ± 41	207 ± 50	NS
Triglyceride (mg/dL)	157 ± 125	153 ± 76	NS
Homocysteine (μmol/L)	19.69 ± 8.51	23.83 ± 11.29	<0.001
Vitamin B12 (ng/L)	705 ± 345	714 ± 337	NS
Folate (μg/L)	5.05 ± 1.89	4.64 ± 2.09	NS

NS = not significant difference, the values are expressed by mean ± standard deviation

Table 2. Comparison of serum homocysteine, vitamin B12 and folate levels between controls and CAD patients.

	Controls (n = 178)	CAD patients (n = 178)	Odd ratio (95%CI)	p value
Increase homocysteine (> 17 μmol/L)	88/178 (49%)	110/178 (62%)	1.65 (1.09-2.52)	0.0249
Low or intermediate B12 (< 200 ng/L)	1/178 (0.5%)	2/178 (1%)	2.01 (0.18-22.30)	0.5620
Low folate (< 3 μg/L)	4/178 (2%)	7/178 (4%)	1.78 (0.51-6.19)	0.5423

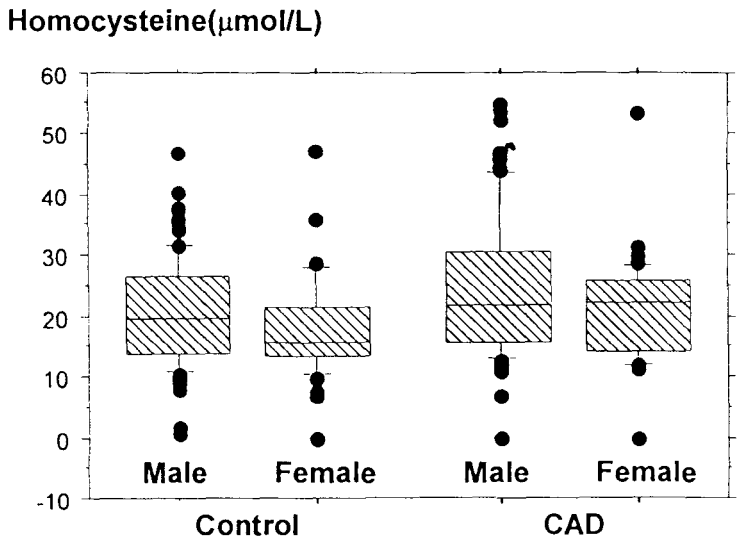

Fig. 1. Serum homocysteine levels in controls and CAD patients.

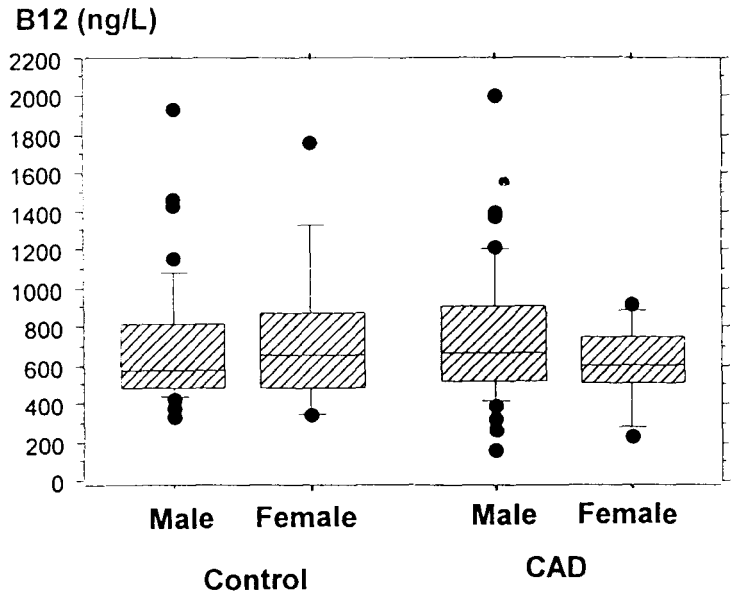

Fig. 2. Serum vitamin B12 levels in controls and CAD patients.

both controls (male: 696 ± 331 ng/L, female: 735 ± 404 ng/L, $p > 0.05$) and CAD patients (male: 744 ± 357 ng/L, female: 595 ± 218 ng/L, $p > 0.05$, Fig. 2). Folate levels were not statistically different between males and females in both controls (male: 4.93 ± 1.93 μg/L, female: 5.53 ± 1.74 μg/L, $p > 0.05$) and CAD patients (male: 4.67 ± 2.26 μg/L, female: 4.45 ± 0.86 μg/L, $p > 0.05$, Fig. 3).

DISCUSSION

The list of preventable and reversible risk factors for atherosclerotic cardiovascular disease continues to grow. Cigarette smoking, high blood pressure, physical inactivity, elevated cholesterol, underlying lipoprotein abnormalities, lipoprotein(a), diabetes, overweight, male gender, and age are well-established risk factors. Many previous studies

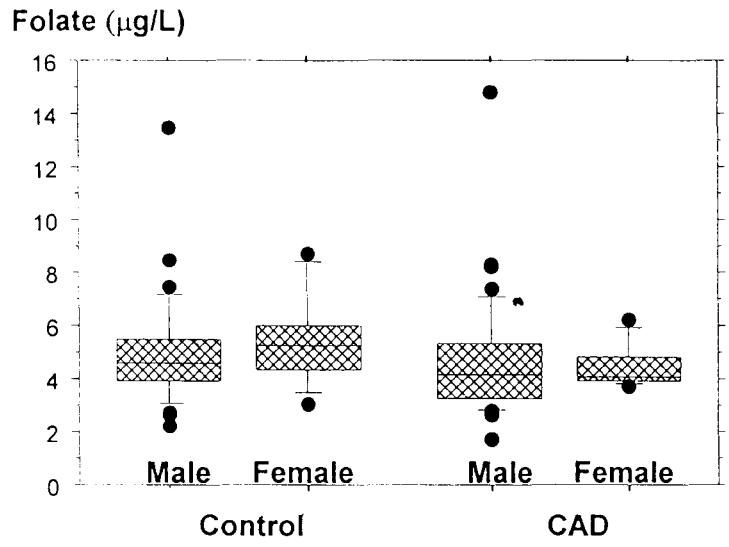

Fig. 3. Serum folate levels in controls and CAD patients.

have demonstrated that elevated plasma homocysteine levels, low plasma folate and low or intermediate vitamin B12 levels were associated with an increased risk of CAD and supplemental folic acid and vitamin B12 can reduce homocysteine levels (4,9-11). Data from the present study confirmed that homocysteine was one of many risk factors in atherosclerotic arterial occlusive disease. In our study, some traditional coronary risk factors were not included. The mean value of total homocysteine (19.69 μmol/L) for the controls was relatively high, but could be best explained by the fact that the controls selected were older than in the reports by other investigators(13). Elevated serum homocysteine above 17 μmol/L were observed in 62 per cent of CAD patients *versus* 49 per cent of the control group. These findings agreed with the reports from previous studies that elevated plasma total homocysteine was observed in 43 per cent of CAD *versus* 15 per cent of the control group(12,13). One prospective, population-based study reported that 31 per cent of controls had levels of plasma total homocysteine above 17 μmol/L(14). Thus, our study confirms that high homocysteine levels are extremely common among the elderly. When we compared the elevation of serum homocysteine between controls and CAD patients, the odds ratio was 1.65 (1.09 – 2.52, p = 0.0249). We could not demonstrate the statistically significant difference between low or intermediate vitamin B12 and low folate with CAD patients. The data also demonstrated that serum homocysteine was almost always higher in men than in women as reported by other investigators (2,3,15). In Thai people, the problem about vitamin B12 deficiency seems to be rare when compared with western countries because of the use of fish sauce in every day cooking. Folate deficiency is also rare in Thai people due to using fresh vegetables for preparing many kinds of food.

Although serum vitamin B12 and folate levels of our population were not below normal, some researchers reported that high homocysteine levels in the elderly were to a large extent related to an inadequate folate, vitamin B12 and vitamin B6 status. High homocysteine levels can be reduced by simple treatment with folic acid, vitamin B12 and vitamin B6 even in the absence of deficiencies of these vitamins. We suggest that when our detects CAD patients who have high levels of serum homocysteine our should start treatment as early as possible in the hope that the risk of myocardial infarction may be further reduced in addition to the reduction of other modifiable risk factors.

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ความสัมพันธ์ระหว่างระดับซีรั่มโฮโมซีสทีน, วิตามิน บี 12, โฟเลท กับผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ในประเทศไทย

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โฮโมซีสทีนเป็นสารกึ่งกลางที่เกิดขึ้นระหว่างเมตาบอลิซึมของเมธไธโอนีนระดับของโฮโมซีสทีนในพลาสม่าขึ้นกับการควบคุมของเอ็นซัยม์ชนิดต่าง ๆ ที่อยู่ภายใต้อิทธิพลของพันธุกรรม, การรับประทานกรดโฟลิค, วิตามิน บี6 และวิตามิน บี12 การเพิ่มสูงขึ้นของซีรั่มโฮโมซีสทีนเป็นหนึ่งในปัจจัยเสี่ยงต่อการเกิดโรคหลอดเลือดแดงโคโรนารี่ การที่จะบอกถึงความสัมพันธ์กันระหว่างระดับซีรั่มโฮโมซีสทีนกับผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ในประชากรไทยนั้นจำเป็นต้องทำการศึกษาแบบ case-control โดยทำการตรวจหาระดับซีรั่มโฮโมซีสทีน, วิตามิน บี12 และโฟเลทในผู้ป่วยโรคหลอดเลือดหัวใจจำนวน 178 รายเปรียบเทียบกับกลุ่มคนปกติจำนวน 178 ราย โดยจับคู่กันทั้งอายุและเพศ ให้ทั้งสองกลุ่มมีค่าใกล้เคียงกันมากที่สุด ในจำนวนนี้เป็นเพศชาย 266 รายและเพศหญิง 90 ราย อายุเฉลี่ยของผู้ป่วยโรคหลอดเลือดหัวใจคือ 60 $\pm$ 10 ปี และอายุเฉลี่ยของกลุ่มคนปกติคือ 58 $\pm$ 10 ปี ซีรั่มโฮโมซีสทีนตรวจโดยวิธี ELISA ส่วนซีรั่มวิตามิน บี12 และซีรั่มโฟเลทตรวจโดยวิธี electrochemiluminescence พบว่าซีรั่มโฮโมซีสทีนในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ (23.83 $\pm$ 11.29 μmol/L) มีค่าสูงกว่ากลุ่มคนปกติ (19.69 $\pm$ 8.51 μmol/L; $p < 0.001$) อย่างมีนัยสำคัญทางสถิติ และยังพบว่าซีรั่มโฮโมซีสทีนในผู้ชายมีระดับสูงกว่าในผู้หญิงอย่างชัดเจนทั้งในกรณีของกลุ่มคนปกติ (ชาย : 20.37 $\pm$ 8.5 μmol/L, หญิง : 17.77 $\pm$ 8.2 μmol/L, $p < 0.05$) หรือในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ (ชาย : 24.91 $\pm$ 11.8 μmol/L, หญิง : 20.73 $\pm$ 8.9 μmol/L, $p < 0.05$) และยังพบว่าระดับซีรั่มโฮโมซีสทีนที่สูงกว่า 17 μmol/L เกิดขึ้นในผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ได้บ่อยกว่าในกลุ่มคนปกติอย่างมีนัยสำคัญ (OR = 1.65, 95%CI = 1.09–2.52, $p = 0.0249$) ไม่พบความแตกต่างของซีรั่มวิตามิน บี12 และโฟเลท ระหว่างผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ และกลุ่มคนปกติ โดยสรุปแม้นว่าวิตามิน บี12 และโฟเลท จะไม่มีความแตกต่างระหว่างสองกลุ่มและไม่ต่ำกว่าเกณฑ์ปกติ การเสริมวิตามิน บี12 และกรดโฟลิคให้กับผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่ก็สามารถลดระดับของซีรั่มโฮโมซีสทีนลงได้และคณะผู้วิจัยหวังว่าแพทย์ผู้ทำการรักษาผู้ป่วยโรคหัวใจดังกล่าวจะให้ความสนใจในการลดปัจจัยเสี่ยงต่อการเกิดภาวะกล้ามเนื้อหัวใจตายเฉียบพลันลงได้

คำสำคัญ : โฮโมซีสทีน, วิตามินบี 12, โฟเลท, ผู้ป่วยโรคหลอดเลือดแดงโคโรนารี่

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