Association between Serum Homocysteine, Folate and B12 Concentration with Coronary Artery Disease in Thai Patients

Worachat Moleerergpoom MD*,
Thunyachai Sura MD**, Piyamitr Sritara MD***

** Division of Cardiology, Police General Hospital*
*** Division of medical genetics and molecular medicine, Faculty of Medicine Ramathibodi Hospital*
**** Division of Cardiology,Faculty of Medicine, Ramathibodi Hospital*

Background : *Hyperhomocysteinemia, associated with low folate and low B12 levels, is known to be an independent risk factor for atherosclerosis. Only a few available data has been demonstrated in Thai patients.*
Objective : *To evaluate serum fasting homocysteine, folate and B12 levels whether to see they are associated with coronary artery disease (CAD).*
Method and Result : *Three hundred and one consecutive suspected CAD patients who underwent coronary angiography at the Police General Hospital were studied. The mean age of the patients, 195 males and 106 females, was 63.0 ± 10.0 year (range 39-85). A total of 218 patients were angiographically demonstrated as having CAD. The mean serum homocysteine level of CAD patients had a non significant higher level than those of 83 non CAD patients: 11.4 ± 6.2 vs 10.2 ± 4.2 umol/L, p = 0.06. Means of folate and B12 level in the CAD patients and non CAD patients were 6.6 ± 4.6 vs 7.0 ± 4.3 nmol/L, p = 0.49 and 650.9 ± 415.4 vs 613.3 ± 443.2 pmol/L, p = 0.56 respectively. No significant correlations were found between homocysteine with folate and B12 levels. Logistic regression analysis showed a significant association between homocysteine and CAD with OR = 1.08 (95%CI, 1.01-1.16), p = 0.03 after being adjusted for age, sex, DM, HT, history of hyperlipidemia, smoking, BMI, folate and B12 levels. No significant association between homocysteine level with the number of coronary vessel stenosis, age, BMI, DM, HT, smoking and history of hyperlipidemia was observed in the present study.*
Conclusion : *Hyperhomocyteinemia, but not folate and B12 levels, may be an independent risk factor for coronary artery disease in Thai patients*

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Homocysteinuria, increased excretion of homocysteine in urine, is a clinical syndrome found in patients presenting with mental retardation, seizures, lens dislocation and premature atherosclerosis[1]. Homocysteine, a non-protein forming sulfur amino acid formed as an intermediate step in metabolism of methionine, can cause vascular injury and a prothrombotic state altered by both inherited and acquired factors. Folate and B12 involved in substrate and cofactors for remethylation of homocysteine to convert back to methionine so nutritional deficiency of these two vitamins are associated with high serum homocysteine[2-5]. Many studies including case-control and prospective studies in different ethnic groups showed independent association between elevated serum homocysteine and atherosclerosis; coronary heart disease[6-9], stroke[10-12], deep vein thrombosis[13], and peripheral vascular disease[14-16]. Only a few studies have been demonstrated in a Thai population. A cross sectional case control study was then performed with the objective to determine whether the serum homocysteine, folate and B12 levels were associated with coronary artery disease and whether there is any relationship between homocysteine and major coronary risk factors in patients proven to have any coronary heart diseases by underwent coronary angiography.

Correspondence to : Moleerergpoom W, Division of Cardiology, Police General Hospital, Bangkok 10330, Thailand.

Material and Method

Study population

A total of 301 patients suspected of having CAD underwent coronary angiography in the Police General Hospital with the following indications: 55.5% with chronic stable angina, 20.7% with unstable angina, 12.5% with preoperative evaluation, 4.7% with cardiomyopathy and 6.6% of others. The study was performed from December 1999 to Febuary 2001. Concents was obtained from all the patients at their first visit which included history taking and complete physical examination. Twelve hours fasting blood samples of all eligible patients were collected at the time the coronary angioghaphy was performed. Patients taking either regular oral vitamins or serum creatinine > 2 mg/dl or malnutrition status were excluded from this study. Conditions defined in patients in the present study followed the standard criterias of WHO; hypertension those who had a history of taking antihypertensive drugs or blood pressure > 140 mmHg systolic and/or > 90 mmHg diastolic, hyperlipidemia those who had a total cholesterol > 200 mg/dl or had a history of taking any lipid lowering drugs, smoking those who were a current smokers or stop smoking for less than 2 years, and coronary heart disease defined by angiographic stenosis at least 50% of lumen diameter in any of the main vessels, left main stenosis without stenosis of the right coronary was classified as two-vessel disease. The control group were patients who had normal coronary angiography or lumen stenosis less than 50% in all vessels involved.

Biochemical Measurments

Fasting plasma of eligible patients was separated from blood cells by immediate centrifugation. Serum was obtained and kept at a temperature of -27 C for analysis serum homocysteine (Hcy), folate and B12 levels by high-performance liquid chromatograhy (HPLC) method (Ubbink 20, 21, 22)

Statistical Analysis

Student's t-test and ANOVA were employed for comparing means between groups. Pearson Correlation was used for determining correlation between homocysteine, folate, B12, age and BMI. The association between groups categorized by variables was determined by Chi square and logistic regression analysis was used for determining association between homocysteine and CAD by adjusting confounding risk factors such as age, sex, BMI, smoking, HT, hyperlipidemia. All analysis were two tailed and p value less than 0.05 was considered significant.

Results

301 patients were eligible for determining serum concentration of homocysteine which had a mean 11.0 $\pm$ 5.7 umol/L and a median = 10.05 umol/L (range 1.5-48.2).

Fig. 1 shows normal distribution of CAD and control group with median = 10.1 umol/L (range 1.5-48.2) and 9.9 mol/L (range 2.75-22.5), respectively.

Table 1 shows the baseline characteristics of patients with CAD and the control group. Mean age of the patients was 63.0 $\pm$ 10.0 years, 195 males and 106 females. The control patients had a higher mean age than those with CAD. Most of CAD patients were male 67.9% vs 57.1% in the control group. There was significantly more diabetes and hyperlipidemia in the CAD group. No significant difference in BMI, HT and smoking was found between the two groups. Mean of homocysteine level in the CAD was slightly higher than that in the control but was not significant, 11.4 $\pm$ 6.2 vs 10.2 $\pm$ 1.2 umol/L respectively, p 0.06. There was also not significantly different means of folate and B12 level in the CAD and Control groups, 6.6 $\pm$ 4.6 vs 7.0 $\pm$ 4.3, p = 0.49 and 650.9 $\pm$ 415.4 vs 613.3 $\pm$ 443.2, p = 0.56.

Means of homocysteine in the CAD were not significantly higher than those in the control group in both the male and female group (Fig. 2). Means of homocysteine in males higher than those of females but also not significant 11.4 $\pm$ 5.9 vs 10.2 $\pm$ 5.1 umol/L,

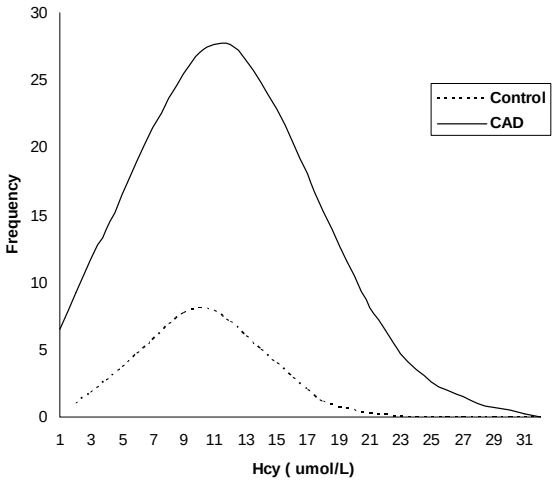

Fig. 1 Normaldistribution of homocysteine in Control & CAD

Table 1. Baseline characteristic of patients and means of homocyteine,folate and B12 in CAD and control groups

Characteristic	CAD (n= 218)	Control (n= 83)	*P* value
Age	62.0 ± 10.1	65.5 ± 9.8	0.005
Sex (M)%	67.9	57.1	0.05
BMI (kg/m^2)	24.3 ± 4.0	24.1 ± 3.6	NS
DM%	39.4	18.2	< 0.0001
HT%	54.6	57.1	NS
Smoking%	20.1	11.7	NS
Hyperlipidemia%	48.2	33.8	0.02
Homocysteine (umol/L)	11.4 ± 6.2	10.2 ± 4.2	NS
Folate (nmol/L)	6.6 ± 4.6	7.0 ± 4.3	NS
B12 (pmol/L)	650.9 ± 415.4	613.3 ± 443.2	NS

p = 0.9.

Correlation between homocysteine vs folate and B12 levels (Fig. 3)

No significant correlations were found between homocysteine vs folate and vs B12 levels with r = -0.001, p = NS and r = 0.072, p = NS respectively.

Association between homosysteine with coronary risk factors and the numbers of vessels stenosis

There was no association between homocysteine and various coronary risk factors such as age, BMI, smoking, DM, hyperlipidemia and HT. Means of homocysteine were not different in the CAD patients according to the number of vessel stenosis; 10.8 ± 4.8, 11.9 ± 6.9 and 11.1± 6.1 umol/L in a single vessel, double vessel and triple vessel disease respectively.

Multivariate Analysis

Fig. 4 shows the logistic regression model for homocysteine, folate, B12 and various factors which could be confounders such as age, BMI, sex, DM, hyperlipidemia, HT and smoking were analyzed as to whether there was independent association with CAD. Homocysteine was weakly significantly associated with CAD with OR = 1.08 (1.01-1.16) p = 0.03. There was also siginficant association between CAD and age, DM and hyperlipidemia in the present study.

Discussion

The average of homocysteine level from population-based survey in various ethnic groups ranged from 7.0-9.6 umol/L[17]. Age, sex and ethnicity

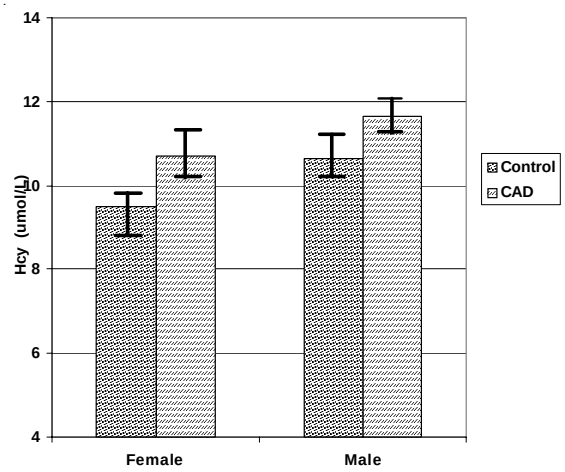

Fig. 2 Means (±SE) of Hcy in CAD and Control according to sex

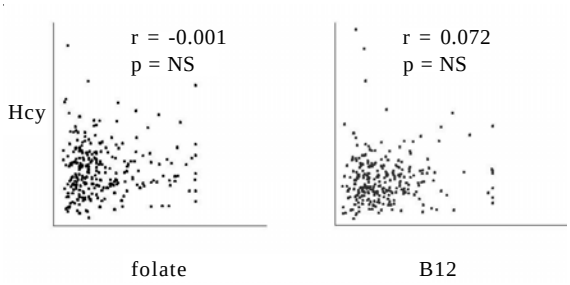

Fig. 3 Correlation between homocysteine vs folate and B12

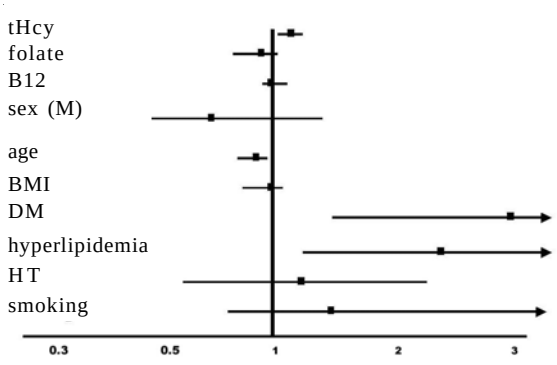

Fig. 4 OR of tHcy, folate, B12 and other risk factors for CAD

were major factors of variation. A review of data from a cross-sectional and retrospective case control study published by Hankey GJ et al[18] demonstrated that the homocysteine level in CAD ranged from 11.3-16.7 umol/L while the control group was slightly lower 8.9-14.7 umol/L. The present data is concordant

with other investigators; however, the present study could not prove a significant different in means of the homocysteine level betweeen these two groups as in many previous reports. The explanation may be the advanced age of the control group. Following the multivariate analysis for adjusting many confounding factors, homocysteine was found to be an independent risk for CAD with OR. 1.08 (1.01-1.16) p = 0.03. The interpretation was every 1 umol/L of homocysteine increment increased risk of CAD by 1.08 folds (or 8%). According to this weak association, can we accept homocysteine to be a new risk factor in Thai ? Larger number of patients need to be explored later but we should concern this new risk factor when atherosclerosis develop in any young or in whom have no other risk factors. Regarding Vitamin B12 and folate, both study groups had comparable Vitamin B12 and folate levels. Use of fish sauce in Thai cooking may be the reason for these similarities as it contains high folate and fresh vegetables are a natural source of Vitamin B12. Due to a small size of the study population, the relationship of homocysteine with age, sex and other coronary risk factors could not be identified. Selhub J, et al proposed to use the level above 95th percentiles of population-based survey to classify hyperhomocysteinemia[17]. Homocystiene data in a Thai population is not available; therefore, the population in the present study could not be classsified. Pathogenesis of homocysteine involved in atherosclerosis is still unclear. Numerous studies explored the sites of adverse effect of homocysteiene induced vascular injury interaction with plasma lipoproteins clotting factors and platelets. Altered vascular endothelium function including proliferation of vascular smooth muscle cells and collagen deposit in plaque was also observed. Folic acid supplementation is the major therapeutic intervention for the treatment of elevated homocysteine levels[19]. Although numerous studies of folic acid supplementation in hyperhomocystinemia have been performed, it remains to be confirmed that homocysteine-lowering therapy will prevent important atherosclerosis vascular events in patients with hyperhomocysteinemia. Several large randomized clinical trials are needs to address this issue.

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ความสัมพันธ์ระหว่าง โฮโมซิสทีน โฟเลต และ วิตามิน บี 12 กับโรคหลอดเลือดหัวใจตีบในคนไทย

วรชาติ โมพีฤกษ์ภูมิ, ธันยชัย สุระ, ปิยะมิตร ศรีธารา

ภาวะ สารโฮโมซิสทีน สูงในเลือด เป็นปัจจัยเสี่ยง ต่อการเกิดโรคหลอดเลือดหัวใจตีบ ซึ่งมักมีความสัมพันธ์กับ ระดับสาร โฟเลต และ วิตามิน บี 12 ในเลือดที่ต่ำ ข้อมูลส่วนใหญ่เป็นข้อมูลที่ได้จากต่างประเทศ จึงทำการศึกษาภาวะนี้ในคนไทย โดยศึกษาผู้ป่วยที่ถูกส่งมาทำการตรวจ สวนหัวใจ จำนวน 301 คน ที่โรงพยาบาลตำรวจ เป็นผู้ชาย 195 คน ผู้หญิง 106 คน อายุเฉลี่ย 63±10 ปี (39-85 ปี) พบว่า มีโรค หลอดเลือดหัวใจตีบ 218 คน และ กลุ่มควบคุม คือ ไม่เป็นโรค มีหลอดเลือดหัวใจ ปกติ 83 คน ค่าเฉลี่ย ของสาร โฮโมซิสทีน ในกลุ่มที่เป็นโรคหลอดเลือดหัวใจตีบเท่ากับ 11.4 ± 6.2 vs. 10.2 ± 4.2 ไมโครโมลต่อลิตร ในกลุ่มควบคุม p=0.06 ค่าเฉลี่ย ของสาร โฟเลต ในกลุ่มที่เป็นโรคหลอดเลือดหัวใจตีบ เทียบกับกลุ่มควบคุม เท่ากับ 6.6 +_4.6 vs. 7.0 ± 4.3 นาโนโมลต่อลิตร p =0.49 และระดับ วิตามิน บี 12 ในเลือด 650.9 ± 415.4 vs. 613.3 ± 443.2 พิโคโมล ต่อลิตร ตามลำดับ p=0.56 ไม่พบความสัมพันธ์ระหว่าง ระดับของ โฮโมซิสทีน กับ โฟเลต และ วิตามิน บี 12 การวิเคราะห์ Logistic regression แสดง ความสัมพันธ์ ระหว่าง ระดับ โฮโมซิสทีน กับ โรคหลอดเลือดหัวใจตีบ มีค่า 0dds ratio เท่ากับ1.08 (95 %CI, 1.01-1.16) p = .03 หลังจากควบคุมโดยปัจจัย อื่นๆ คือ ระดับ สาร โฟเลต วิตามิน บี 12 อายุ เพศ เบาหวาน ความดันโลหิตสูง ประวัติไขมัน ในเลือด สูบบุหรี่ มวลรวม สรุปว่า ภาวะ โฮโมซิสทีนสูง ในเลือด อาจเป็นปัจจัยเสี่ยงต่อการเกิดโรคหลอดเลือดหัวใจ ตีบในคนไทย