Hyperhomocysteinemia in Patients with Coronary Artery Disease

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Abstract

Hyperhomocysteinemia has been recognized as a risk factor of atherosclerosis. This study was aimed to measure the risk of coronary artery disease in patients with hyperhomocysteinemia. Age, HDL level, tHcy level and history of DM were independent risk factors for coronary artery disease. The level of tHcy of 11.0 mmol/L provides the best sensitivity and specificity of predicting coronary artery disease.

Key word : Hyperhomocysteinemia, Coronary Artery Disease, Atherosclerosis

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Homocysteine (Hcy) is a sulfur containing amino acid formed during metabolism of methionine. The methionine and homocysteine's metabolism depends on vitamins B_2, B_{12} and folate as co-factors[1]. Many inherited disorders of methionine metabolism which result in elevation of total plasma homocysteine (tHcy), i.e. hyperhomocysteinemia, are associated with early development of atherosclerosis and occlusive disease in arteries and veins[2-7]. Recent reports have shown that even mild hyperhomocysteinemia is a common, independent, easily modifiable causal risk factor for car-

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diovascular disease which may be of equal importance to hypercholesterolemia, hypertension and smoking[8-10]. Many factors including diet, lifestyle and environmental factors play an important role in regulation of plasma homocysteine level[11]. Our group previously reported that hyperhomocysteinemia was associated with coronary artery disease[12]. The threshold of total plasma homocysteine concentration which confers risk of developing atherosclerosis is unknown. It has been reported that a fasting plasma homocysteine level of greater than 10 mmol/L can impair endothelial function[13].

Thus, the proposal of this study was to 1) measure the risk of coronary artery disease in patients with hyperhomocysteinemia 2) find the fasting total plasma homocysteine level which best predicts patients with coronary artery disease in the Thai population.

MATERIAL AND METHOD

Study population

The authors recruited 135 patients who underwent coronary angiography at Her Majesty Cardiac Centre, Faculty of Medicine, Siriraj Hospital and had more than 50 per cent diameter stenosis of at least one coronary and compared the risk factors to 537 healthy individuals who came for routine annual check up in our laboratory during the same period. Both case and control subjects gave informed consent for blood sampling. The control subjects were free of any overt vascular disease. There was no attempt to match cases and controls for sex, age or any other risk factors.

Study measurements

Demographic variables and history of risk factors were obtained by questionnaires which were reviewed for completeness by trained nurses and cardiologists prior to blood sampling.

Blood for tHcy determination was taken after at least 10 hours fasting with the subjects seated, for those with coronary artery disease it was taken prior to coronary angiography. All samples were immediately centrifuged at 3000 g for 15 minutes and transported on ice and stored at $-70^{o}C$ within one hour. Measurement of tHcy level was performed by fluorescence polarization immunoassay (FPIA) technique with IMx system from Abbot Laboratory. Internal and external coefficient of variation for tHcy in the laboratory were 2.98 per cent and 3.58 per cent respectively.

Definition of variable

Current smoking was defined as the use of any tobacco within a week of blood sampling. Subjects were defined as hypertensive if they had a history of having systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$95 mmHg at least in two separate occasions or were on antihypertensive medication. Hypercholesterolemic was defined as serum cholesterol $\geq$240 mg/dl or if a cholesterol-lowering drug was being prescribed by a physician. Diabetes mellitus was diagnosed if patients had a history of fasting plasma glucose $\geq$140 mg/dl on two occasions or if they were taking any oral hypoglycemic agent or treated with insulin as well as those patients and subjects who had fasting plasma glucose >140 mg/dl from a blood sample taken during the survey.

Statistical analysis

The authors calculated mean and SD for continuous and percentage for categorical variables. Case–control differences were tested with the Student's unpaired t-test, Pearson's chi-square or Fisher exact tests where appropriate. Binomial logistic regression analysis was used to identify predictors of having coronary artery disease. Odds ratio (OR) were calculated for all risk factors and different tHcy levels.

Using levels of tHcy from both case and control groups we calculated sensitivity and specificity of different tHcy levels and used Receiver Operating Curve (ROC) to detect which was the best tHcy level that can predict coronary artery disease. An OR or difference between groups was said to achieve statistical significance if the probability value was 0.05 or less.

RESULTS

Characteristics and risk factors of coronary artery disease

There was significant mismatch in case and control groups in all parameters. (Table 1) The 537 subjects in the control group were very healthy and younger with a higher percentage of females compared to 135 patients with coronary artery disease. There was a higher frequency of positive history of all risk factors in the disease (case) group. However, there was no significant difference in the fasting cholesterol level. There was elevated triglyceride, low density lipoprotein (LDL) and tHcy

Table 1. Demographic information of controls and patients with coronary disease (CAD).

	Control (n=537)	CAD (n=135)	OR	p-value
Age (yr)	32 + 5.9	62 + 9.7	-	<0.001
Male (%)	244 (45%)	99 (73%)	3.3	<0.001
History of elevated cholesterol	56 (16%)	71 (53%)	9.6	<0.001
History of diabetes mellitus	6 (1%)	56 (42%)	63.3	<0.001
History of hypertension	29 (6%)	78 (58%)	23.7	<0.001
Smoking history	152 (29%)	55 (41%)	1.7	0.006

Table 2A. Results of fasting plasma lipids and tHcy levels.

	Control	CAD	p-value
Cholesterol level (mg/dL)	209.5 ± 37.7	207.6 ± 51.3	0.685
Triglyceride (mg/dL)	98.1 ± 63.3	149.8 ± 74.1	<0.001
LDL (mg/dL)	126.4 ± 34.2	139.3 ± 45.8	0.003
HDL (mg/dL)	64.2 ± 23.2	38.4 ± 11.0	<0.001
tHcy (mmol/L)	9.05 ± 3.80	16.19 ± 6.60	<0.001

Table 2B. Number (%) of patients with different tHcy and lipid levels.

	Control	CAD	OR	p-value
Cholesterol >200 mg/dL	308 (57%)	72 (53%)	0.85	0.399
Cholesterol >240 mg/dL	108 (20%)	31 (23%)	1.18	0.465
LDL >130 mg/dL	236 (44%)	76 (56%)	1.64	0.01
HDL <35 mg/dL	7 (1.3%)	60 (44%)	0.017	<0.001
tHcy >11.0	135 (25%)	106 (79%)	11.7	<0.001
tHcy >12.0	93 (17%)	94 (70%)	11.2	<0.001
tHcy >15.0	25 (4.7%)	61 (45.5%)	17.1	<0.001

Table 3. Univariate and multivariate analysis of risk factors for CAD.

Risk Factors	OR	95% CI of OR	p-value
Multivariate			
Age	1.5	1.27-1.76	<0.001
HDL level (mg/dL)	0.84	0.77-0.92	0.0002
tHcy level (mmol/L)	1.40	1.13-1.73	0.0017
History of DM	13.70	1.16-162.02	0.0059

levels in the case group compared to the control group (Table 2). Using all the variables in Tables 1 and 2 we calculated the OR by the binomial logistic regression analysis stepwise technique in order to find independent factors that were associated with coronary artery disease (Table 3). History of diabetes mellitus was consistently the strongest factor associated with the disease. Patients with more advanced age, low HDL levels and elevation of tHcy were more likely to have atherosclerosis of the coronary arteries. Risk of having the disease also increased with increasing tHcy level (Fig. 1).

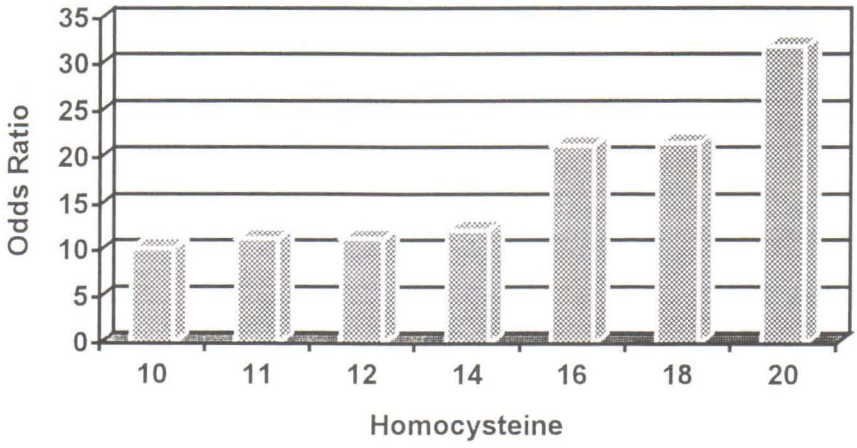

Fig. 1. Dose-response relationship between tHcy level and CAD risks.

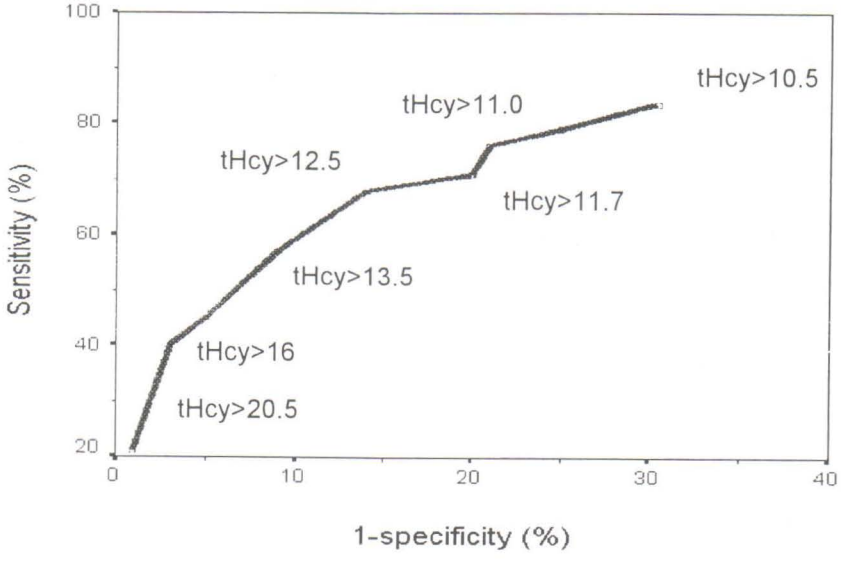

Fig. 2. Receiver Operating Curve (ROC) of tHcy at various levels.

The ROC curve (Fig. 2) shows no clear cut level of tHcy which will provide the best prediction of patients with coronary artery disease. However, from Table 4, it can be seen that higher tHcy levels were more specific to the disease than lower tHcy levels. The tHcy levels at 60th and 75th had a sensitivity between 84 per cent to 68 per cent of detecting coronary artery disease.

DISCUSSION

In this study, we observed that patients with coronary artery disease were more likely to have elevation of tHcy level compared to a healthy population similar to what was previously reported (2-7,14). It was known that advanced age and male sex were associated with elevation of tHcy levels (14). Despite the mismatch of age and sex in our

Table 4. Sensitivity and specificity of detecting CAD at different levels of tHcy.

Percentile	tHcy level (mmol/L)	Sensitivity %	Specificity %
50	9.6	90.3	59.4
60	10.5	83.6	69.6
65	11.0	79.1	74.9
70	11.7	70.9	80.4
75	12.5	67.9	86.0
80	13.5	56.7	90.9
85	15.0	45.5	95.3
90	16.0	39.6	97.0
95	20.5	20.9	99.1

case and control groups, using the multivariate analysis model, we found that advanced age, low HDL, history of diabetes mellitus and elevated tHcy level were found to be independent risk factors for coronary artery disease. Patients with coronary artery disease had significantly higher levels of LDL and triglyceride with lower HDL level when compared to the control subjects. The indifference between levels of fasting cholesterol in both groups (Table 2A) in spite of a significant history of hypercholesterolemia (Table 1) in the disease group probably reflects treatment received of this group. However, male sex, history of hypercholesterolemia and elevated LDL and triglyceride were not independent risks in our studied population.

We also demonstrated (Fig. 1) that an increasing level of tHcy was associated with the increasing risk of having coronary artery disease as previously reported[15-19].The increasing risk can be seen from the tHcy level of 10 mmol/L. The ROC curve (Fig. 2) failed to show a clear point at which tHcy level would be best to predict patients with coronary artery disease. However, from Table 4, it is suggested that tHcy of 11.0 mmol/L (65^{th} percentile) was the best value which gave the highest sensitivity and good specificity of predicting the disease. This level is lower than levels used in other reports as the cut off point for the abnormal level[14,15,18.19].

Limitation of this study

The major limitation of our study was the mismatch of age and sex between both studied groups. Both factors have been reported to have association with elevation of tHcy and coronary artery disease[7,14]. Moreover, other factors which may effect tHcy levels such as dietary intake, physical activity, and alcohol consumption were not included in our analysis. Furthermore, the presence of coronary artherosclerosis was confirmed by coronary angiography in the disease group, but in the control groups no angiography was used to confirm those without disease.

SUMMARY

Despite the limitation, we have demonstrated that elevation of tHcy levels is as strong a risk factor as other risk factors of having coronary artery disease. Our data also indicated that increasing level of tHcy was associated with increasing risk of the disease. We propose that a tHcy level of 11.0 μmol/L is the best level to use in screening subjects who are suspected of having coronary atherosclerosis.

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ภาวะ hyperhomocysteinemia ในผู้ป่วยโรคหลอดเลือดหัวใจ

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ภาวะระดับ homocysteine ในเลือดสูง เป็นปัจจัยเสี่ยงต่อการมีภาวะหลอดเลือดแข็งตัว การศึกษานี้ได้ดูความสัมพันธ์ของระดับ homocysteine ในเลือด ในผู้ป่วยที่มีโรคหลอดเลือดหัวใจ และพบว่าภาวะ hyperthomocysteinemia เป็นปัจจัยเสี่ยงต่อการมีภาวะหลอดเลือดหัวใจแข็งตัวเช่นเดียวกัน อายุ, ระดับ HDL ต่ำและประวัติการเป็นเบาหวาน โดยที่ระดับ homocysteine ที่ 11.0 mmol/L ให้ความไวและความจำเพาะในการทำนายโรคดีที่สุด

คำสำคัญ : Hyperhomocysteinemia, โรคหลอดเลือดหัวใจ, โรคหลอดเลือดแข็งตัว

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