CORRELATION BETWEEN TOTAL PLASMA HOMOCYSTEINE LEVEL AND GRADING OF CORONARY ARTERY DISEASE

S. J. Mirhoseini[1], H. Radmehr[1, 2], M. Sanatkarfar[1, 4], S. Kh. Froozan-Nia[3], M. Salehi[1], M. GH. Alemohammad[1], A. R. Bakhshandeh[1] and M. Baalbaki[4]

1) Cardiovascular Surgery Department, Imam Khomeini Medical Center, Tehran University of Medical Sciences, Tehran, Iran

2) Iranian Tissue Bank, Tehran University of Medical Sciences, Tehran, Iran

3) Cardiovascular Surgery Department, Afshar Hospital, Yazd University of Medical Sciences, Yazd, Iran

4) Anesthesia Department, Besat Hospital, Hamedan University of Medical Sciences, Hamedan, Iran

Abstract- Elevated total plasma homocysteine (tHcy) levels constitute a risk factor for coronary artery disease (CAD). A possible relationship was investigated between admission plasma homocysteine level and the angiographic severity and extension of coronary artery disease in patients with CAD. This study looks at the relationship between total plasma homocysteine and severity of coronary artery disease. From April 2006 to December 2006, 100 consecutive patients (65 male and 35 female) that referred to our institute for coronary artery bypass graft surgery enrolled. Fasting blood samples for homocysteine were obtained on admission. Plasma homocysteine concentration was measured with high-performance liquid chromatography (HPLC). Our patients presented in Group 1, total plasma homocysteine >12 micromoles per liter and Group 2, total plasma homocysteine =<12 micromoles per liter. Vessel score assessed the number of vessels with significant stenosis and grading of atherosclerosis (Extent Score) was intended to assess the atherosclerotic involvement of the entire arterial length and circumscribe. Our study was shown age > 60 years was correlated with high tHcy, but gender, hypertension, history of smoking, hypercholesterolemia, family history, and diabetes mellitus were not statistically difference between two groups. A positive correlation was found between abnormal plasma homocysteine level and vessel score (r = 0.35; p=0.002). Moreover, a positive correlation was also found with extent score (r = 0.46; p =0.002). As results of these scoring, there was a better correlation between the tHcy level and the extent of CAD when compared with the vessel score (r = 0.68, $p < 0.001$). Abnormal elevated homocysteine levels in patients with coronary artery disease correlated with the extent of atherosclerotic disease.

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INTRODUCTION

Atherosclerotic plaques are the most common cause of CAD (1).

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Corresponding Author: Mehdi Sanatkarfar, Department of Cardiovascular Surgery, Imam Khomeini Medical Center, Tehran, Iran
Tel: +98-21-66925901
Fax: + 98 21 6929977
Email: mehdi_sanatkar@yahoo.com

More than 200 coronary risk factors have been reported. Recently homocysteine has been shown to be involved in the pathogenesis of CAD.

Homocysteine is a sulfur-containing amino acid produced by demethylation of the essential amino acid methionine (2). Homozygotes with homocystinuria have high levels of circulating tHcy (> 100 μmol/l) are at high risk for premature arteriosclerotic vascular disease and venous thrombosis (3, 4). If homocystinuria remains untreated, about 50% of patients may experience

thromboembolic events and mortality could reach 20% before the age of 30 years (4) Observations in patients with homocystinuria led to the idea that tHcy may be involved in the pathogenesis of arteriosclerosis. Observations in approximately 80 clinical and epidemiological studies suggested that elevated tHcy is a risk factor for atherosclerotic vascular disease and for arterial and venous thromboembolism (5). Although the exact mechanism of atherothrombosis associated with hyperhomocysteinemia is not clearly understood, in many of the reported effects of plasma homocysteine are thought to be mediated by its atherogenic effects, such as vascular smooth cell migration and proliferation (5); and prethrombotic properties, such as inhibition of thrombomodulin activity, (6) reduction of protein C activation (6, 7) the increase of platelet aggregation (8) and predisposition to endothelial cell injury (9). The aim of this study was to examine a possible relationship between admission plasma homocysteine level and the extent of coronary artery disease in patients candidate for coronary artery bypass graft surgery (CABG).

MATERIALS AND METHODS

From April 2006 to December 2006, 100 consecutive patients (65 male and 35 female) that have been candidate for CABG and referred to Cardiovascular Surgery Department, Imam Khomeini Medical Center, Tehran University of Medical Sciences were enrolled. Every patient completed a detailed questionnaire providing information about history of hypertension, hyperlipidemia, smoking, diabetes mellitus and family history of premature CAD (documented CAD in at least one first-degree relative before the age of 55 years for men and 65 years for women).

Exclusion criteria for patients and controls included renal dysfunction (serum creatinine > 133 µmol/l), thyroid or psychiatric disease, anticonvulsant therapy, megaloblastic anaemia, pregnancy and malignancy. Patients with a recent MI (< 2 weeks), or subjects who were taking multivitamins were also excluded.

Venous blood was obtained the day after admission. Serum was prepared by centrifugation at 1000 g, at 4 °C up to 30 min after collection.

Plasma total homocysteine, which includes the sum of protein-bound and free homocysteine, was measured by high-performance liquid chromatography with fluorescence detection (10, 11) Plasma homocysteine was recorded in units of micromoles per liter. Our patients presented in Group 1, total plasma homocysteine >12 micromoles per liter and Group 2, total plasma homocysteine ≤12 micromoles per liter. Vessel Score assessed the number of vessels with significant stenosis (> 70%) with a score ranging from O to 3 depending on the number of vessels involved. Grading of atherosclerosis (Extent Score) was intended to assess the atherosclerotic involvement of the entire arterial endothelium was as followed: segmental atherosclerosis involvement of the arterial (Grade 1) and diffuse atherosclerosis involvement (Grade 2), assessed by angiographic evidence and intraoperative assessment.

Statistical Analysis

Analyses were performed using statistical programma SPSS version 11.0 for Windows. Categorical variables are expressed as percentage and continuous variables as mean ±standard deviation. Student's *t* test was used in comparisons of continuous variables between two groups. For categorical variables, a chi-square test was used to test differences between study groups. ANOVA was used in comparisons of values among subgroups of group 1. Pearson correlation coefficient was used to evaluate the linear association between the plasma homocysteine level and vessel score and extent of CAD.

RESULTS

Baseline characteristics of groups 1 and 2 are given in Table 1. 77 patients (77%) had plasma homocysteine > 12 micromoles per liter (Group 1). The mean age of patients was 61.2+/-22.4 (38 to 78 years). 41 patients in group 1 and 8 patients in group 2 had age>60 years (p<0.05).

Our study was shown gender, hypertension, history of smoking, hypercholesterolemia, family history, and diabetes mellitus were not statistically difference between groups.

Table1. Baseline characteristics of group 1 (tHcy> 12 µmol/l) and group2 (tHcy<=12 µmol/l).

Variables	tHcy>12 µmol/l	tHcy<=12 µmol/l	*p*
Age (>60 years)	41	8	0.04
Sex (male)	38	16	0.64
Diabetes Mellitus	14	6	0.58
Hyperlipidemia	5	2	0.84
Hypertension	24	5	0.18
Familial history of CAD	17	4	0.29
Smoking	17	8	0.23
Grading of atherosclerosis			
I	44	22	0.002
II	33	1	
Number of vessel disease			
1VD	3	1	0.002
2VD	7	8	
3VD	54	12	
Left main	13	2	

One-, 2-, 3-vessel and left main disease was found in 3 (3.9%), 7 (9%), 54 (70.2%) and 13 (16.8%) of patients in group 1, and 1 (4.3%), 8 (34.8%), 12 (52.2%) and 2 (8.7%) of patients in group 2 respectively. A positive correlation was found between plasma homocysteine level and vessel score (r = 0.35; *p*=0.002).

Similar correlation was also found with extent score (r = 0.46; *p*=0.002) from traditional methods. But, the most significant correlation was determined with extent scoring method (r = 0.68, $p < 0.001$) (Fig. 1, 2).

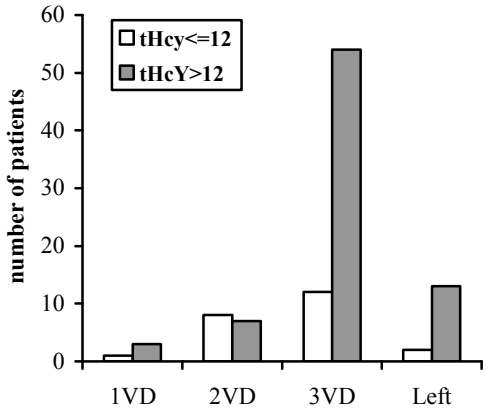

Fig. 1. Correlation between tHcy level (µmol/l) and number of vessel disease (Vessel Score).

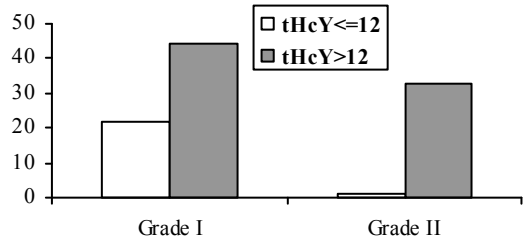

Fig. 2. Correlation between tHcy level (µmol/l) and grading of atherosclerosis (Extent Score).

DISCUSSION

Identification of new markers associated with an increased risk of CAD may provide a better insight into the pathology of coronary atherosclerosis and facilitate the development of preventive and therapeutic measures (12). To the best of our knowledge, this is the first study demonstrating the significant correlation between plasma homocysteine levels, which is an independent risk factor for CAD and vessel score and grading of atherosclerosis in patients that candidate for CABG. Previous studies demonstrated a significant ($p < 0.001$) univariate association between tHcy and CAD (13, 14).

Ten of 13 case-control studies that assessed the association between fasting tHcy levels and CAD showed significantly higher levels of tHcy in patients with CAD compared to those without CAD

(15-27). Furthermore, seven of the 13 case-control studies adjusted the tHcy levels for CAD risk factors and six of them observed an increased risk of CAD with elevated fasting tHcy after this adjustment (16, 18, 23-27). The European study from 19 centres concluded that increased plasma tHcy levels confer an independent risk of vascular disease similar to that of smoking or hyperlipidemia (25).

The results of the Multiple Risk Intervention Trial failed to find any association between CAD and tHcy (27). In a review of 43 studies, Christen observed that, in contrast to cross-sectional and case-control studies, prospective studies indicated less or no predictive ability for tHcy in cardiovascular disease (28). Therefore, whether tHcy is an independent risk factor for CAD remains controversial. We found that tHcy levels were associated with the vessel score and extent of CAD. Hyperhomocysteinemia may promote atherosclerosis and thrombosis by a number of possible mechanisms. First, oxidation of Hcy to disulphides generates superoxide anion radicals and hydrogen peroxide, resulting in inactivation of nitric oxide (NO) and endothelial cell dysfunction that may contribute to vasospasm, thrombosis, and progression of atherosclerosis (29).

Second, hyperhomocysteinemia may result in irreversible homocysteinylation at epidermal growth factor-like domains in fibrillin-1 and in many other extracellular proteins of the coagulation-anticoagulation and lipoprotein transport pathways, with subsequent malfunctioning of such pathways (30). Furthermore, metabolic conversion of Hcy to thiolactone may play a role in Hcy-induced vascular damage (31). Third, Hcy and oxidized LDL-C forms LDL-Hcy aggregates, which are precursors of the foam cell, cholesterol and lipid deposits within developing atherosclerotic plaques (32). These aggregates enhance platelet adhesion to endothelial cells by inducing tissue factor activity (by Hcy) and up-regulating intercellular adhesion molecule-1 (ICAM-1) by oxidized LDL (33).

Fourth, hyperhomocysteinemia may increase factor VIIa, cause the generation of thrombin, inhibit protein C activation and may down-regulate thrombomodulin (18). Finally, Hcy has also been seen to induce DNA damage (35, 36).

Interactions between prothrombotic factors, hypofibrinolysis and dyslipidemia may play an important pathogenic role in premature CAD (36, 37).

Incidence of tHcy>12 μmol/l in our study was more compared to other studies (36). This inconsistency of our finding might be due to mean of tHcy in patient's candidate for CABG higher compared to patients with CAD established by angiography that showed in previous study (36). Moreover, genetic and different nutrition habits in our country assign to developed countries could also be define this difference.

Al Obaidi and associates showed that increased homocysteine levels are associated with a higher risk of ischemic myocardial injury in patients presenting with acute coronary syndromes (38). In a study by Stubbs and associates, moderately increased plasma total homocysteine concentration measured on admission is a strong predictor of late adverse cardiac event in patients with acute coronary syndromes (39). Some studies support the hypothesis that elevated plasma tHcy levels and low folate are independent risk factors for MI among young women (35) and that elevated tHcy is a strong predictor of late cardiac events in acute coronary syndromes (39), therefore early diagnosis and treatment of hyperhomocysteinemia in patients with CAD are very important.

We failed to show any association between risk factors of CAD (diabetes mellitus, hypertension, hyperlipidemia, familial history and smoking) and tHcy levels. This is probably due to small sample size. This underlines need for larger prospective and intervention studies to define the association of tHcy and CAD. Two methods were used to determine the relationship between extent of CAD and tHcy levels in our study. In the first method, an analysis of the relationship between tHcy levels and the number of vessels (Vessel Score) with significant stenosis was carried out, and a positive correlation was observed ($r = 0.35$; $p=0.002$). The second method, used in this study was grading of atherosclerosis (Extent Score). In this method, the extension of atherosclerotic disease in the coronary artery tree was explored ($r = 0.46$; $p=0.002$). As results of these scoring, there was a better correlation between the tHcy level and the

extent of CAD when compared with the vessel score ($r = 0.68$, $p < 0.001$). In recent studies by Schynder and Bozkurt, increased tHcy level is associated with severely narrowed major coronary arteries (40, 41). We also found this relationship in our study.

Plasma homocysteine levels can easily be decreased with folic acid or vitamin B6, B12 supplementation. Several randomized studies are currently underway to assess the effects such therapy on progression of angiographic extent of CAD.

We concluded that tHcy is associated with vessel score and extent of CAD in patients candidate for CABG and further studies are required to assess the effect of homocysteine-lowering treatment on the vessel score and extent of CAD.

Conflict of interests

We have no competing interests.

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