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Original Article

Association of Hyperhomocysteinemia with Coronary Artery Disease in Southern Iran

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Abstract

Background: Differences in the prevalence and impact of hyperhomocytenemia on vascular disease between countries and races have been reported. Most studies have been undertaken in North American and European populations and the importance of plasma total homocysteine (tHcy) level as a risk factor for coronary artery disease in the Middle East particularly in Iran, however, is not known.

Objective: To determine the association of hyperhomocysteinemia with coronary artery disease in Shiraz population, a city in the South West of Iran.

Method: A case-control study was conducted in 195 men with angiographically defined coronary artery disease and 201 healthy controls. Plasma tHcy concentrations were analyzed in baseline samples by high performance liquid chromatography.

Results: Geometric mean of plasma tHcy was significantly higher in patients (10.6±5.2 μmol/l) than in control (7.9±3.1 μmol/l) group ($p<0.001$). Comparing the top fifth (quintile), of the plasma tHcy distribution to the bottom fifth, the adjusted odds ratio of coronary artery disease was 2.3 (95% CI 1.0–5.5) and there was a positive trend of increasing risk across quintiles of the plasma tHcy distribution ($p=0.02$).
Conclusion: High plasma tHcy is associated with coronary artery disease in Iranians living in Shiraz.
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Keywords • Homocysteine • coronary artery disease • Shiraz

Introduction

Homocysteine is a sulfur-containing amino acid, the plasma concentration of which is determined by genetic factors and nutritional deficiencies of vitamins B6, B12 and folic acid.[1] Plasma tHcy is increasingly recognized as an independent risk factor for vascular disease in different populations.[2-4] Plasma tHcy concentrations above the 80th percentile of normal have been reported in almost 40% of patients with vascular diseases, including coronary artery disease (CAD).[3] In a recent report of a meta-analysis of 20 prospective studies involving 3820 participants, it was found that lowering plasma tHcy concentrations by 3 μmol/l from its current levels would reduce the risk of ischemic heart disease by 16% (11% to 20%), deep vein thrombosis by 25% (8% to 38%), and stroke by 24% (15% to 33%).[4] So far, differences in the

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Table 1: Characteristics of patients and control groups

Characteristics	Patients (n=195)	Controls (n=201)	*p* value
Age (year)	59.6 ±11.3	52.9± 10.2	0.07
Hypertension (%)	31.2	12.3	0.004
HDL cholesterol (mmol/l)	0.68± 0.17	0.72± 0.21	0.03
Total cholesterol (mmol/l)	6.5±1.4	4.3 ±0.6	0.01
LDL (mmol/l)	4.3 ±1.5	3.1 ± 0.2	0.02
TG (mmol/l)	2.5 ± 0.8	1.4 ± 0.4	0.01
Hypercholesterolemia %	48.0	15.2	0.002
Current smoking (%)	25.0	11.3	0.001
Plasma tHcy (μmol/l)			
Geometric mean	10.6 ± 5.1	7.9 ± 3.1	<0.001
Mean	11.4 ± 4.9	8.4± 3.1	<0.001

prevalence and impact of hyperhomocyteinemia on vascular diseases among countries and races have been reported.[5-6] Most previous studies, however, have been undertaken in North American and European populations, but the importance of plasma tHcy levels as a risk factor for CAD in the Middle East, particularly in Iranians is not studied. This study, therefore, describes plasma tHcy distribution and its association with CAD in a sample of male adults living in Shiraz, southern Iran.

Patients and Methods

This study was approved by ethical committee of Shiraz University of Medical Sciences and a written consent was obtained from each individual. A case-control study was carried out on 195 male patients with angiography-proven CAD, considered as patient group, and 201 healthy male individuals served as control group. The city of Shiraz was geographically divided into 14 areas according to the first three digits of the telephone numbers. Telephone numbers were chosen at random from each area and a total of 560 individuals were asked to attend our laboratory at the Biochemistry Department, Shiraz University of Medical Sciences for blood sampling. Out of 560 individuals, 461 attended and after filling a questionnaire, 201 individuals met the inclusion criteria. Patients with CAD were chosen from the cardiology outpatient department of Faghihi Hospital affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. Criteria for CAD were angiographically proven as shown by more than 50% stenosis in one or more major epicardial vessels apparent on multiple projections. At the time of enrolment, relevant data on past medical history, current smoking habits, alcohol consumption and drug therapy were obtained from all participants. Exclusion criteria for both patients and control group included cardiomyopathy, psoriasis, blood disorders, cancer or chronic renal failure and drug therapy that are known to interfere with homocysteine metabolism (*e.g.,* phenytoin, carbamazepine, methotrexate, pencilinamine, etc.). Tobacco consumption was defined as current smoking. Participants with serum cholesterol levels ≥6.5 mmol/l were considered hypercholesterolemic. Individuals were considered hypertensive if their systolic blood pressure was ≥160 mm Hg or diastolic blood pressure ≥95 mm Hg.

Samples for plasma tHcy were taken after an overnight fasting, placed on ice, centrifuged within 1 hr of sampling, and the separated plasma was stored at -70˚C before assay. Additional fasting serum samples were collected for total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglyceride (TG). Total serum cholesterol, HDL cholesterol and TG were determined using enzymatic photometry on Cobas autoanalyzer (USA). Plasma tHcy were analyzed using high performance liquid chromatography as described elsewhere.[7] The inter-assay coefficient of variation for determination of Hcy in plasma was less than 7.2%.

Statistical Methods

The results of the variables are presented by means ±standard deviation. Student t test and Chi square analysis were used to compare the significant differences of the variables between cases and controls.

The distribution of plasma tHcy was positively skewed**.** Therefore, logarithmic transformations were used and geometric mean was calculated. Spearman's correlation coefficients used to examine the association between plasma tHcy and potential confounding cardiovascular factors such as age, hypertension, LDL, HDL, cholesterol and smoking. Determination of quintiles (fifths) of plasma tHcy concentrations were based on the plasma tHcy distribution in the control group. The top fifth of tHcy distribution and the logistic regression model were used to calculate odds ratios and their 95% confidence intervals for coronary heart disease. The logistic regression analysis

Table 2: Relative risk of major coronary artery diseases across quintiles of plasma tHcy distribution

	Odds ratio (95% confident intervals)		
Plasma tHcy (μmol/l)	**Crude**	**Age-adjusted**	**Multivariate adjusted**[a]
<9.4	1	1	1
10.2-	1.3 (0.52 to 2.9)	1.1 (0.47 to 2.8)	1.0 (0.41 to 2.5)
11.9-	1.5 (0.64 to 3.4)	1.4 (0.53 to 3.2)	1.1 (0.47 to 2.8)
13.2-	1.7 (0.76 to 3.8)	1.6 (0.7 to 3.6)	1.5 (0.7 to 3.2)
15.3	2.3 (1.1 to 5.0)*	2.2 (1.0 to 4.8)*	2.3 (1.0 to 5.5)*
p Trend	0.002	0.002	0.02

*p<0.05

[a] Adjusted for blood pressure, LDL, HDL,TG, cholesterol and current smoking

was used to adjust odds ratios for cardiovascular risk confounders. All reported p values are two tailed.

Results

The clinical and biochemical characteristics of the patients and control groups are summarized in Table 1. The geometric mean of plasma tHcy concentrations was significantly higher in patients (10.6±5.2 μmol/l) than in control group (7.9±3.1 μmol/l). The number of current smokers, hypertensive and hypercholesterolemic among the patients group were also significantly higher than the control group. Serum HDL levels were significantly lower in patients than in control groups. Results from Spearman's correlation coefficients showed that plasma tHcy was positively associated with age (r=0.35, p=0.001) and diastolic blood pressure (r=0.2, p=0.04). Plasma tHcy, however, did not correlate strongly with plasma TG, smoking, total, HDL, and LDL cholesterol. Table 2 shows the crude and adjusted odds ratios of CAD across quintiles of plasma tHcy distribution. Individuals who were in the top fifth of plasma tHcy had more than 2-fold increased risk of CAD than those in the lowest fifth. Adjustments for age and other cardiovascular confounders had little effect on odds of CAD.

Discussion

Results from this study suggest that high plasma tHcy is associated with CAD in Iranians. This association appears to be independent of other confounding factors such as cholesterol, HDL, LDL, TG and smoking. The results from odds ratios of CAD for individuals across quintiles of tHcy concentration, as shown in Table 2, suggest that the CAD risk factor for individuals in the top fifth of tHcy was more than 2-fold of the individuals in the lowest fifth. After adjustment for all confounders first for age and then for other confounders the increased risk of CAD was still more than 2-fold when compared with the individuals in the lowest fifth.

The risk of CAD in the fifth quintile of plasma tHcy remained significant when adjusted for age and other confounding factors (Table 2). Various case-control studies have reported an association between plasma tHcy and CAD .[1-4] This study confirms that plasma tHcy is an independent risk factor for CAD for the Iranian population living in the southern part of the country. The results of earlier population studies examining the relation between tHcy and CAD have been conflicting.[8-11] Differences in design, analytical approach as well as limitations of statistical power may, therefore, account for, at least, part of the variations in the findings of the populations' studies. However, it is possible that the differences in nutritional or genetic factors may modify the strength relations between plasma tHcy and coronary heart disease in the different study populations.

Further studies are needed to evaluate the contribution of tHcy to coronary risks in women and to define the relative importance of environmental and genetic determinants of tHcy in our population.

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