Coronary Heart Disease

Association of Serum Homocysteine and Coronary Heart Disease in an Iranian Urban Population

Fatemeh Bandarian, Hossein Fakhrzadeh, Ramin Heshmat, Masoumeh Nouri and Bagher Larijani

Background: Several studies have shown that elevated serum homocysteine levels are associated with an increased risk of coronary heart disease (CHD). This survey was designed to investigate the association between hyperhomocysteinemia and CHD in an Iranian urban population.

Methods: In a cross-sectional study, 358 residents (129 men and 229 women) of the 17th District of Tehran, Iran aged 25-64 years old (mean 44.08 ± 11.77 years) were assessed for ischemic heart disease (IHD) according to the Rose questionnaire and resting electrocardiographic (ECG) analysis by Minnesota code, and CHD risk factors (including serum homocysteine) were compared between ischemic and non-ischemic groups.

Results: Crude prevalence of hyperhomocysteinemia in this population was 53.4%. Mean plasma total homocysteine (tHcy) in men was significantly higher than in women (P = 0.001). There was no significant difference in mean serum vitamin B12, homocysteine and folate between the ischemic and non-ischemic groups (P = 0.31, 0.16 and 0.51, respectively).

Conclusion: According to the results, high serum level of tHcy by itself is not a CHD risk factor in a healthy population, but it should be considered for reduction in CHD patients.

Key Words: Coronary heart disease • Electrocardiography • Homocysteine

INTRODUCTION

Homocysteine (Hcy) is an essential aminoacid in humans. It has been known as a novel and independent risk factor for coronary heart disease (CHD).1-4 The prevalence of hyperhomocysteinemia varies between 5% and 30% in the general population.5

In Iran, the burden of cardiovascular disorders, especially CHD, is high, and they are the leading cause of mortality in the country.6 Nearly 317 out of every 750 daily deaths in 2003 were attributed to cardiovascular disease, and it was estimated that on each day, 2726 years of life had been lost to cardiovascular disease.6

Genetic factors, smoking, hypertension, serum creatinine, total cholesterol and protein and nutritional factors such as vitamin B6, B12 and folate deficiency determine serum total homocysteine (tHcy) concentrations.2

It has been shown that elevated serum Hcy levels are associated with an increased risk of ischemic heart disease (IHD) and stroke.3,7,8 Also, higher Hcy concentrations in IHD or stroke patients than in controls has been reported.3,9,10 Some prospective and case-control studies with inconsistent results, some with highly significant results,10,11 and others with no association have been observed.4,12,13

A recent study has indicated that increase in homocysteine and other novel risk factors have been associated with an increase in Framingham risk score in elderly people.14 A meta-analysis in 1995 showed that a 1 μmol/L increase in Hcy concentrations was associated with a 10% increase in CHD risk;8 in another study 25% reduction in Hcy levels caused 11% decrease in the risk of IHD.15
According to the results of two studies, it has been suggested that tHcy is an independent predictor of mortality in stable and acute CHD.4,16

Our previous studies demonstrated higher prevalence of hyperhomocysteinemia and lower vitamin B12 and folate levels in our population than other studies,17 and we also found a significant association between hypertension and high serum tHcy concentration.18 Due to high prevalence of hyperhomocysteinemia and CHD in our country,6,18 assessment of the association between CHD and homocysteine in this population is important because by its modification, we may prevent high mortality and morbidity due to CHD and therefore reduce CHD cost.

Inspite of several studies, hyperhomocysteinemia has not been accepted yet as an established cardiovascular risk factor and remains controversial. Therefore, we aimed to investigate the association between hyperhomocysteinemia and IHD and to determine the role of homocysteine as a risk factor for IHD.

MATERIALS AND METHODS

This cross-sectional study is a part of “Cardiovascular Risk Factor Survey” in the Population Lab Region of Tehran University of Medical Sciences which was designed based on the methodology of MONICA (Multinational Monitoring of Trends and Determinants in Cardiovascular Diseases)/WHO (World Health Organization) project19,20 by EMRC (Endocrinology and Metabolism Research Center), Tehran University of Medical Sciences (TUMS). The study protocol was approved in TUMS. Three hundred fifty-eight residents of the 17th District of Tehran, Iran between 25 and 64 years old were recruited in the study. They were selected by single-stage cluster random sampling from a group of 255,337 people in 115 clusters. After obtaining informed consent, a structured questionnaire including demographic information and medical history and risk factors was completed for each participant by trained interviewers and physical examination including anthropometric measurements was done. Personal and lifestyle information were obtained by using modified MONICA questionnaires.19

A venous blood sample was taken in overnight fasting state for laboratory tests. The plasma samples were put in a cooled container and immediately sent to the EMRC laboratory, where the plasma was separated within 2 hours of sampling by centrifuge (20 min, RT, at 2000 rpm) and aliquots were stored at -70 °C until performance of assay. Plasma tHcy concentrations (the sum of Hcy and homocysteine-cysteine mixed disulfides, free and protein-bound) were determined on frozen samples by HPLC (high-performance liquid chromatography) method (KNAUER, Germany), coupled with fluorescence detector. The method has been validated over a linearity range of 1-100 µmol/L from plasma. The intra-assay and inter-assay coefficients of variation for Hcy concentrations were 6.9% and 6.1%, respectively. The inter-assay coefficients of variation for folate and vitamin B12 were 7.5% and 6.8%, respectively. We defined hyperhomocysteinemia (HHcy) as Hcy ≥ 15 µmol/L.1 Serum folate levels less than 11 nmol/L and vitamin B12 concentration less than 185 pmol/L considered as vitamin deficiency.

A twelve-lead electrocardiogram (ECG) was obtained from all participants in the resting position. Electrocardiograms were interpreted according to the Whitehall criteria of the WHO Multinational Program for Diabetes and Coronary Heart Disease by two experienced cardiologists.21 Diagnosis of angina pectoris was made by Rose criteria.22 CHD was defined as angina pectoris (grade I or II of Rose criteria), myocardial infarction (possible MI, i.e. major Q wave (Minnesota code 1.1), or history of previous MI and ischemic resting electrocardiographic abnormalities including probable and possible ischemic heart diseases. Probable ischemic heart disease/ECG included major Q or QS wave (Minnesota code 1.1, 1.2) or complete left bundle branch block (Minnesota code 7.1.1). Possible ischemic heart disease/ECG included small Q or QS wave (Minnesota code 1.3), ST depression (Minnesota codes 4.1-4.3), or T-wave items (Minnesota codes 5.1-5.3). ECGs that did not fulfill any of these criteria were categorized as normal. Each individual with any of these criteria was included only once.

Statistical analysis

For interpretation of data SPSS software (version
11.5 for Windows) was used, and $X^2$-test, and independent $t$-test were applied for finding the associations and correlations and comparing the biochemical parameters between the two groups. Multiple Logistic Regression model was used to adjust the data for confounding factors. The results were shown as mean $\pm$ SD, and $P < 0.05$ was considered significant.

RESULTS

Of 358 participants, 36% (129) were male and 64% (229) were female. Their mean age was 43.4 $\pm$ 11.3 years. Table 1 shows the demographic characteristics and biochemical parameters of the study participants. Crude prevalence of hyperhomocysteinemia in this population was 53.4%. Geometric mean of $t$Hcy in men was significantly higher than in women ($20.28 \pm 1.56$ vs. $13.59 \pm 1.49$, 95% CI: 5.3-10.5).

There was no significant difference in mean serum vit B12, $t$Hcy and folate between the ischemic and non ischemic groups (Table 2). Yet after adjusting for confounding factors including age, sex, BMI, smoking and cholesterol using Multiple Logistic Regression model, the relation remained nonsignificant. Analysis of data using two different cut-off points for Hcy (10 and 15 $\mu$mol/L) was done separately, and again, no significant association was found. Hcy quartiles were put in to the Multiple Logistic Regression model, but no significant relation was seen again (data not shown).

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<td>Gender</td>
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<td>Total cholesterol (mean $\pm$ SD) (mg/dl)</td>
<td>197.26 $\pm$ 45.19</td>
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<td>HDL (mean $\pm$ SD) (mg/dl)</td>
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<td>LDL (mean $\pm$ SD) (mg/dl)</td>
<td>102.20 $\pm$ 31.67</td>
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<td>TG (mean $\pm$ SD) (mg/dl)</td>
<td>183.38 $\pm$ 107.80</td>
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<tr>
<td>Diabetes n (%)</td>
<td>54 (12.4%)</td>
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<td>Smoking n (%)</td>
<td>18 (6.64%)</td>
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<td>Geometric mean Folate (mean $\pm$ SD) (nmol/L)</td>
<td>3.97 $\pm$ 1.68</td>
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<td>Geometric mean Vitamin B12 (mean $\pm$ SD) (pmol/L)</td>
<td>258.26 $\pm$ 1.80</td>
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<tr>
<td>Geometric mean Homocysteine (mean $\pm$ SD) ($\mu$mol/L)</td>
<td>15.76 $\pm$ 1.59</td>
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<td>BMI (kg/m2)</td>
<td>28.13 $\pm$ 4.76</td>
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<td>Total cholesterol (mean $\pm$ SD) (mg/dl)</td>
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<td>HDL (mean $\pm$ SD) (mg/dl)</td>
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<td>LDL (mean $\pm$ SD) (mg/dl)</td>
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<td>102.91 $\pm$ 28.54</td>
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<td>TG (mean $\pm$ SD) (mg/dl)</td>
<td>206.08 $\pm$ 137.99</td>
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<td>Diabetes n (%)</td>
<td>21 (23.10%)</td>
<td>33 (9.60%)</td>
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<td>Smoking n (%)</td>
<td>2 (4.00%)</td>
<td>15 (6.80%)</td>
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<td>Geometric mean Folate (mean $\pm$ SD) (nmol/L)</td>
<td>3.98 $\pm$ 1.81</td>
<td>3.97 $\pm$ 1.65</td>
<td>0.51</td>
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<tr>
<td>Geometric mean Vitamin B12 (mean $\pm$ SD) (pmol/L)</td>
<td>265.86 $\pm$ 1.86</td>
<td>255.95 $\pm$ 1.78</td>
<td>0.31</td>
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<tr>
<td>Geometric mean Homocysteine (mean $\pm$ SD) ($\mu$mol/L)</td>
<td>16.72 $\pm$ 1.75</td>
<td>15.54 $\pm$ 1.54</td>
<td>0.16</td>
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<tr>
<td>BMI (kg/m2)</td>
<td>28.97 $\pm$ 5.17</td>
<td>28.08 $\pm$ 4.66</td>
<td>0.18</td>
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</table>

* Significant difference.
There was no significant relationship between hyperhomocysteinemia, vit B12 and folate deficiency with ischemic criteria including possible ischemia, probable ischemia, possible MI, angina pectoris and total ischemia.

Mean serum Hcy in smokers was significantly higher than in non-smokers ($P = 0.004$), and serum folate was significantly lower in this group ($P = 0.04$). Also, serum vit B12 was higher in smokers, but it was not significant ($P = 0.06$).

We did not observe any significant difference in mean serum Hcy, vit B12 and folate between subjects with metabolic syndrome and without it as well as obese subjects and non-obese and also diabetics and non-diabetics (data not shown).

Sex-specific analysis revealed no significant difference in mean Hcy, vit B12 and folate between ischemic and non-ischemic women or men (data not shown).

**DISCUSSION**

This study investigated the association between hyperhomocysteinemia and IHD. No significant relationship was found between serum tHcy levels and IHD according to the ECG findings, which agree with the findings of Retterstol et al.\textsuperscript{23} and other previous studies, which didn’t observe any association between serum tHcy concentrations and major cardiac events.\textsuperscript{12,13,24}

However, several studies have shown association between plasma tHcy concentrations and CHD. They have reported elevated plasma tHcy as an independent risk factor for CHD.\textsuperscript{3,4,25,26} These findings were supported by the results of a meta-analysis which indicated that tHcy is a modest predictor of IHD and stroke risk in a healthy population independent of traditional cardiovascular risk factors.\textsuperscript{27} Nygard et al. study could only show a weak relationship between tHcy and angiographic findings,\textsuperscript{4} while other studies revealed a positive association between plasma tHcy and risk of severe coronary atherosclerosis according to angiography results.\textsuperscript{28}

Our findings are the same as those of Nikkari et al. who did not find significant difference in serum tHcy between men with angina pectoris and controls.\textsuperscript{29} Nikkari et al. observed higher serum tHcy in men with previous myocardial infarction than in controls results which have been repeated in several previous studies \textsuperscript{5,10,29} but in the present study, although tHcy in men was higher than in women in the study population, it was not elevated in the people with ischemic heart disease.

Retterstol et al., investigating a cohort of young CHD patients, similar to our findings also could not see association between serum tHcy and IHD. They showed that tHcy was only a predictor of total and cardiac mortality in older ages.\textsuperscript{23} The correlation between tHcy and folate and age which was seen in our study was confirmed also in their study.\textsuperscript{73} Although in the present study tHcy levels increased by age, in one report from Asia (India), it was higher in younger-age subjects.\textsuperscript{26}

Our findings also are supported by some clinical trials that found that Hcy-lowering treatment with vitamin B and folic acid had no secondary prevention role in acute myocardial infarction. One of these studies showed that administration of folic acid, vitamin B6, and vitamin B12 for 5 years did not reduce the incidence of major vascular events in high-risk patients with vascular disease (including cardiovascular causes and myocardial infarction).\textsuperscript{30,31}

The study population of the present investigation was apparently healthy (without any history of severe CHD or other chronic disease) and relatively young individuals (not prone to CHD) and this may have caused us not to see any significant association between CHD and hyperhomocysteinemia. These are two limitations of the present study. Having no gold standard such as angiography to confirm CHD is another limitation of our study. Also, in this study, plasma was separated within 2 hours of sampling, which is too long and may have influenced the results of homocysteine. So, it is suggested to separate plasma from whole blood within 30 min by centrifugation (10 min, 4 °C at 2500 rpm). Up to now, different surveys have reported various results. The difference of this study with previous ones is the study method. Most of the previous studies have compared the Hcy levels between patients with established CHD diagnosis and controls, but we investigated CHD according to ECG findings in a healthy population (and excluded participants with severe CHD) with normal and high serum homocysteine concentrations, which rarely has been examined previously, so the different results may be explained by this. However, future cohort and case-controlled studies with larger sample size are required to confirm these results in this region.
According to our results, serum level of tHcy by itself is not a risk factor for CHD in a healthy population, but it should be considered for reduction in CHD patients.

REFERENCES