PLASMA HOMOCYSTEINE LEVELS AND CORONARY HEART DISEASE RISK IN JORDANIAN SUBJECTS

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ABSTRACT

Objective: To examine the association between hyperhomocysteinemia and the risk of coronary heart disease and to highlight the relation between hyperhomocysteinemia and other risk factors of coronary heart disease including smoking, hypertension, and hypercholesterolemia.

Methods: A total of 45 patients with coronary heart disease and 35 healthy controls of either sex, aged 60 years or less, were examined. Blood samples were obtained from all subjects at fasting and 4 hours after a methionine-loading test. The risk for hyperhomocysteinemia and its relation to other risk factors were examined by logistic regression analyses.

Results: Sixty percent of the patients had hyperhomocysteinemia (fasting and postload) as compared to 40% of the controls. The odds ratio for coronary heart disease in patients with elevated fasting and postload homocysteine was 1.85 (C.I = 1.3-2.5, p=0.00) and 1.24 (C.I =1.1-1.39, p=0.00) respectively. No interaction between hyperhomocysteinemia and other conventional risk factors was observed. The likelihood of a coronary heart disease event increased approximately by 2-fold in patients with elevated fasting homocysteine levels, and by 1.24-fold in those with elevated postload homocysteine levels. Only smoking and hyperhomocysteinemia were strong predictors for coronary heart disease among our study group.

Conclusion: Hyperhomocysteinemia is significantly and independently associated with coronary heart disease in our Jordanian sample.

Key words: Homocysteine (Hcy), Coronary Heart Disease (CHD), Hyperhomocysteinemia, Methionine Loading Test.

JRMS June 2004; 11(1): 7-16

Introduction

The major risk factors of coronary heart disease (CHD) have been identified in both retrospective and prospective studies. However, hyperlipidemia, hypertension, smoking, diabetes mellitus, obesity, and alcohol intake are all known CHD risk factors. To date, many studies indicate that hyperhomocysteinemia is a very well recognized risk factor for CHD [(1)].

Homocysteine (Hcy), a sulfur- containing amino-acid, is formed in the metabolic pathway of methionine that is obtained from either plant or animal origin proteins in the diet. It is also referred to as a metabolite [(2)] or as an intermediate of methionine [(3)]. In humans, homocysteine is remethylated to methionine by folate and cobalamine dependent enzymes, and catabolized to cysteine by vitamin B_6 dependent enzyme. Thus, any disturbance in the metabolic pathway of methionine, due to genetic defect and/or nutrient deficiencies, leads to Hcy accumulation in the circulation [(4)].

Many studies have shown that moderate hyperhomocysteinemia is associated with increased risk of coronary atherosclerosis, thrombosis, cerebrovascular disease, and peripheral vascular

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Manuscript received October 14, 2002. Accepted July 2, 2003

disease. Verhoef *et al*. reported that elevated plasma Hcy is an independent risk factor for myocardial infarction [5].

Hyperhomocysteinemia can be caused by other environmental determinants that may be more frequent than some genetic factors [6]. These determinants include nutrient deficiencies of folate, vitamin B_6, and vitamin B_{12}, alcohol intake, smoking, chronic diseases, increasing age and being a male [7].

The role of moderate hyperhomocysteinemia as an independent risk factor for CHD has been recently supported in many case-control studies [8]. Information on nutrition and health status of the Jordanian population is relatively scanty.

To date, only one study has been conducted to investigate hyperhomocysteinemia as a risk factor for CHD in an Arab population [9]. However, it has been revealed that elevated level of Hcy is an independent risk factor for CHD among Arab men in Syria [9]. Furthermore, the identification of all possible risk factors for CHD is critical in disease prevention and therapy in high-risk populations.

The information obtained from the present study will help in evaluating the role of hyperhomocysteinemia in CHD mortality and morbidity. In addition, results from this study will provide a baseline data for further studies.

Finally, the knowledge gained from this study will be helpful in CHD prevention and therapy, particularly, in high-risk individuals.

The purpose of this study was to investigate the relation between tHcy and CHD risk in Jordanian subjects, as well as the relation between hyperhomocysteinemia and other conventional risk factors.

Methods

Subjects and Data Collection

In the present case-control study, 45 patients known to have CHD of either sex and 35 healthy controls were examined. All subjects were recruited from King Hussein Medical Center in Amman, Jordan during the whole year 1999. The protocol approval was obtained from both Howard University Institutional Review Board (IRB) and the Protection of Human Subjects Ethics Committee in Jordan. Patients who were diagnosed during the previous year and confirmed to have clinical evidence of CHD were considered eligible to participate in the study. Patients with a history of chronic diseases such as: renal disease, thyroid disease, alcoholism, diabetes mellitus, cancer, and/or patients on medications that are known to interfere with total homocysteine (tHcy) metabolism; and pregnant women were excluded from the study. Systematic sampling was used for the selection of patients. The controls were selected from either healthy personnel in the center or their relatives. All participants in the present study were Jordanians. Informed written consent was obtained from all subjects who agreed to participate in the study. Data were collected through blood samples and a questionnaire. Bostom *et al*. indicate that measurements of plasma fasting tHcy alone do not detect cases that might have post methionine load (PML) hyperhomocysteinemia. Hence, in the present study, blood samples were obtained from all subjects at fasting (before a methionine load) and 4 hours after a standardized methionine load [10].

Three conventional risk factors were examined in the present study including smoking, hypertension, and hypercholesterolemia. Smoking habit was determined at the time of CHD diagnosis for patients and at the time of methionine loading test for the controls. Blood pressure readings were taken for all subjects before and after the methionine-loading test; and the average of four readings was taken. Information on the usage of hypertensive drugs was obtained from either the medical records (patients) or directly from the subjects (controls). Cholesterol levels for the patients were obtained from the medical records. Blood samples were obtained from the controls at fasting and were analyzed for serum cholesterol levels on the first day of participation.

The questionnaire provided information on age, gender, ethnic group, height, weight, smoking, and vitamin intake. The form was completed on the first day of participation.

Definition of Variables

Age was analyzed as both a continuous and a categorical variable. Plasma tHcy level above the 75^{th} percentile of the controls distribution [11] was regarded as hyperhomocysteinemia 12.3 (μmol/L) for fasting tHcy, and 33.6 (μmol/L) for postload tHcy. Controls with tHcy levels below the 75^{th} percentile of the controls distribution were used as reference in odds ratio estimates. Cholesterol levels were analyzed as either a continuous or a categorical variable. Cholesterol levels of > 200 mg/dL were considered as hypercholesterolemia. Subjects with cholesterol levels < 200 mg/dL were used as reference in odds ratio estimates. Since cholesterol distribution was similar among cases and controls, the 75^{th} percentile of controls distribution was also used as a cutoff point (> 220 mg/dL). The odds ratio

estimates for smoking were based on a comparison between current smokers of 20 cigarettes per day and nonsmokers. Smoking was also analyzed as a categorical variable in which current smokers were compared to ex-smokers (who had quit smoking for at least six months before participating in the study) and nonsmokers. Hypertension was considered present if the systolic blood pressure was (160 mm Hg, diastolic blood pressure (95 mm Hg, or if the subject was taking antihypertensive medication [(1)]. According to the previous definition, only two subjects had high blood pressure in which both of them were on antihypertensive drugs. Therefore, the odds ratio estimates for hypertension were based on a comparison between subjects taking medication and those who were not (Yes/No) as a reference.

Biochemical Measurement

High performance liquid chromatography (HPLC) with fluorescence detection was used to determine plasma tHcy levels. Sample preparation was performed as described by Araki and Sako [(12)]. Since the isocratic HPLC system was more feasible in the laboratory, the mobile phase was performed as described by Accinni *et al* [(13)]. All prepared blood samples for tHcy analysis were transferred to the laboratories of Jordan University of Science and Technology (JUST), Irbid, Jordan.

Statistical Analysis

Mean values and standard deviation of the risk factors for CHD were determined. Case-control differences were examined using analysis of variance (ANOVA) and t-test for continuous variables, and chi square for categorical variables. To examine the association between tHcy levels and other risk factors, both bivariate and multivariate analyses were performed. Odds ratio estimates of fasting and postload tHcy levels were performed by logistic regression analyses. The odds ratio estimates plus 95% confidence intervals (CI) are presented for tHcy levels as a continuous variable [(14)]. The odds ratio was also estimated for tHcy as a categorical variable defined by the 75th percentile of controls distribution. Controls with tHcy levels below the 75th percentile of the controls distribution were used as reference. To determine whether tHcy concentrations interact with other conventional risk factors, multiple logistic regression models were performed. Two-tailed p values of 0.05 were considered significant. The statistical analyses were performed using the SPSS Graduate Pack 9.0 for Windows.

Results

Characteristics of the Subjects

The major characteristics of the patients and the control subjects are presented in Table I. Age and gender distribution did not differ between patients with CHD and the controls. The prevalence of hypertension, cholesterol levels, and smoking habits were all similar among cases and controls. The mean level for systolic blood pressure was not significantly higher in controls than in cases (p=0.06). The prevalence of hypertension was 29% in patients and 31% in controls. Among cases, 38% of the patients had elevated cholesterol levels (> 200 mg/dL) as compared to 31% of the controls. Although 60% of the patients were current smokers versus 46% of the controls, the difference between the two groups was not significant (p= 0.08). Pearson correlation tests showed that age was directly and significantly associated with smoking (r =0.3, p= 0.008) and systolic blood pressure (r = 0.27, p= 0.01). Systolic blood pressure was positively associated with cholesterol levels (r = 0.24, p= 0.02) and body mass index (BMI) (r = 0.23, p= 0.04). The adjustment for age and gender did not affect the associations.

The distribution of fasting and postload tHcy levels was shifted toward higher values in patients as compared to controls. Postload tHcy levels were positively and significantly associated with fasting tHcy levels (r=0.73, p= 0.00) and the increase in tHcy as well (r=0 .8, p= 0.04).

The mean fasting and postload tHcy levels were 29% and 16%, respectively, higher in patients than in controls (p<0. 05). The total increase in tHcy was also examined (postload minus fasting). The increase in tHcy did not differ among the subjects.

A total of 60% of cases had hyperhomocysteinemia as compared to 40% of controls. Postload tHcy levels was identified in 6 (7.5%) more subjects (patients and controls) who were not classified by fasting tHcy levels. Similarly, the increase in tHcy was identified in 4 (5%) additional subjects (patients and controls) who were not identified by either fasting or postload tHcy levels.

Plasma Homocysteine and other Risk Factors

The relationship between tHcy concentrations and each risk factor was examined by bivariate analysis (Table II). Hypertension, cholesterol levels, and smoking were significantly related to homocysteine levels (p < 0.05) in patients with low risk profiles. Homocysteine levels did not differ between males and females. Age was not significantly related to

Hcy levels (p=0.09). Patients with cholesterol levels less than 200 mg/dL had significantly higher tHcy levels as compared to their respective controls (p= 0.01). Similarly, patients who were normotensives (p= 0.01) and nonsmokers (p=0.02) had significantly higher tHcy levels as compared to controls. Plasma tHcy levels were not significantly higher among smokers, as compared to controls (p= 0.08). However, when all risk factors were included in the model, only hypertension and smoking remained significant (p < 0 .05). No interaction effect was found between tHcy and the conventional risk factors, even after controlling for age and gender (age and gender were included in the model as covariates).

Relative Risks for Elevated tHcy

The odds ratios for patients with and without elevated fasting and postload tHcy levels were determined by single logistic regression analyses. In these analyses, both fasting and postload levels were significantly related to CHD risk (Table III). Among patients with elevated fasting tHcy levels, the odds ratio for tHcy levels was 1.85 [(1.3-2.5), p= 0.00] as compared to subjects below the 75th percentile of the controls distribution. For postload tHcy levels, the odds ratio was 1.24 [(1.1-1.4), p= 0.00] as compared to the reference group.

For patients without fasting and postload elevated tHcy levels, the odds ratio for tHcy levels was decreased but remained a strong predictor for CHD (p <0 .05).

The odds ratio for tHcy levels was examined with the presence of each risk factor separately.

In the presence of all risk factors including hypertension, hypercholesterolemia and smoking, the odds ratio for patients with elevated fasting tHcy levels were 3.0 [(1.5-5.9), p=0. 001] as compared to the reference group; and 1.14 [(1.03-1.26), p= 0.007] for smokers of 20 cigarettes per day or higher as compared to nonsmokers. Adjustment for age and gender did not affect the significance of the prediction. In this analysis, hypercholesterolemia, and hypertension were not significantly related to CHD (Table IV). Since the results did not show any statistical significance, only the combined, age and sex-adjusted results are presented. However, when adjusted for the presence of smoking, the odds ratio for fasting tHcy levels were reduced but remained significant predictors of CHD [odds ratio =1.9 (1.3-2.6), p=0 .0001].

Similarly, in patients with elevated postload tHcy, the odds ratio for tHcy levels was 1.3 [(0.17-3.6), p= 0.0002)] as compared to subjects with tHcy levels below the 75th percentile of controls distribution; and 1.0 [(1.0-1.16), p=0.01] for smokers of 20 cigarettes per day as compared to nonsmokers (Table IV). The relationship between tHcy levels and the risk for CHD was examined. Fig. 1 illustrates the continuous relationship between fasting and postload tHcy levels with the risk of CHD in patients as compared to controls. The estimated odds ratio for CHD per 5-µmol/L increments in tHcy was 2.1 [(1.2-3.8), p=0.0008]. Finally, no interaction effect was observed between fasting or postload tHcy levels and the conventional risk factors.

Discussion

Our findings demonstrate the association between tHcy concentrations and CHD risk among Jordanian subjects. It is believed that fasting tHcy concentration is an indicator of poor remethylation pathways resulting from either genetic defects and/or folate and cobalamine deficiencies. The postload methionine level reflects cystathionine β synthase (CBS) impairments, a vitamin B_6 dependent enzyme [(6)].

A significant increase in either fasting or postload tHcy ranges 10-30% in patients as compared to controls and was between 13 case-control studies [(11)]. The mean fasting tHcy level was 29% higher in patients than in controls (p=0.003). Among patients, 44.4% had elevated fasting tHcy levels as compared to 25.7% of controls (p= 0.03). Our results were consistent with the results of other studies.

Bostom *et al* indicated that using fasting tHcy levels alone in determining hyperhomocysteinemia may result in misclassification of more than 40% of subjects who may have postload hyperhomocysteinemia [(10)]. Graham *et al* reported that a further 27% of patients with hyperhomocysteinemia were identified by a postload methionine test [(1)].

In our study, fasting tHcy levels identified the majority of the hyperhomocysteinemia patients. Only three (6.6%) additional patients were identified by postload tHcy concentrations. Although the mean increase in tHcy (postload minus fasting) levels did not differ between patients and controls, approximately 6% of the patients with elevated tHcy levels who were not identified by either fasting or postload, were identified by this measure. Fasting and postload levels were correlated (r=0.7, p=0.00). The mean for postload tHcy was 16% higher in patients than in controls (p= 0.02) (Table I).

The prevalence estimates of the major risk factors for CHD in Jordan in 1991 were 32% for hypertension, 46% for hypercholesterolemia, and 48% and 10% for smoking among males and females, respectively [15]. In our sample, the prevalence of hypertension was 30%, 36.2% for hypercholesterolemia, and 54% for smoking. The differences in major risk factors of CHD between patients and controls were not as expected. The prevalence of hypertension and hypercholesterolemia did not differ between patients and controls. Smoking habit was non significantly more prevalent among patients (60%) as compared to controls (46%) (p=0.08).

The association between tHcy levels and other conventional risk factors were examined. Age and gender are known to influence tHcy levels. Plasma tHcy levels tend to be higher among older male subjects. Genders differences may be related to hormonal factors and/or muscle mass [3]. Our results did not show a significant age-sex relationship with tHcy concentrations. Plasma tHcy levels were not significantly higher in patients older than 50 years as compared to their respective controls.

Plasma tHcy levels increase with age and are higher among males than females. Due to the fact that age and gender differences are related to diet and vitamin status [8], the contradiction in our findings cannot be explained in the absence of vitamin measures.

Many studies have addressed the association between hyperhomocysteinemia and the conventional risk factors. Graham *et al* reported interaction events between tHcy and two risk factors [1]. Elevated tHcy levels interacted strongly with both hypertension and smoking. The joint effect was most pronounced among women as compared to men.

Results from a recent study showed that fasting tHcy levels tend to be significantly higher among hypertensive Chinese subjects ($p< 0.05$) [16].

Verhoef *et al* reported a positive correlation between high blood pressure and tHcy levels in patients with CHD as compared to controls [17]. Nygard *et al* illustrated positive correlation between elevated tHcy levels and other risk factors including age, gender, smoking, hypertension, and hypercholesterolemia [18].

Glueck *et al* reported significant tHcy interaction effects with high-density lipoprotein (p=0.012) and triglycerides (p=0.02) in atherosclerotic patients with hyperlipidemia [19]. Hoogeveen *et al* reported an interaction between hyperhomocysteinemia and type 2 diabetes to risk of 5-year mortality [20]. The odds ratio for hyperhomocysteinemia was 2.5 (1.07-5.91) in diabetics as compared to 1.3 (0.87-2.06) in non diabetics (p for interaction = 0.08).

In our study, fasting tHcy levels were highly related to hypertension and smoking, but not hypercholesterolemia (P value < 0.05). In these models, fasting tHcy levels were significantly higher among normotensive patients as compared to controls (p= 0.01). Similarly, fasting tHcy levels were more pronounced among patients with normal cholesterol levels (< 200 mg/dL) and nonsmokers ($p<0$ .05) (Table II). Results from a recent study showed that the association between tHcy levels and MI was stronger among subjects with normal hypertension as compared to hypertensives [21].

Interestingly, when these risk factors were examined among patients with elevated fasting tHcy levels (12.3 (μmol/L)), only the prevalence of smoking was significantly higher among patients as compared to their respective controls (p=0.01).

Moreover, the association between postload tHcy and other risk factors was also examined. Among the risk factors, only smoking was significantly associated with postload tHcy levels (p= 0.01). The association between postload tHcy and CHD was independent of smoking. The postload levels were significantly higher among patients as compared to controls irrespective of their smoking habits (p< 0.05). Multiple regression analyses did not show interaction events between hyperhomocysteinemia and other risk factors.

Lack of association between elevated tHcy levels and both hypertension and hypercholesterolemia that was observed in this study may be related to the predominance of patients with normal hypertension and cholesterol levels, or possibly due to the similarity among patients and controls in terms of these two risk factors. In addition, the 1999 WHO criteria for the definition of hypertension was not used, another text criterion was used instead [1] which may provide another explanation for the observed association between hyperhomocysteinemia and hypertension.

Serfontein *et al* reported a significant association between decreased vitamin B_6 levels and smoking [22]. The authors suggested that these results might provide another explanation by which smoking may be involved as a CHD risk factor. Accordingly, the highly significant association that was observed in the present study between smoking and postload tHcy level could be related to vitamin B_6 status since postload tHcy level is an indicator for CBS, a vitamin B_6 dependent enzyme.

The odds ratio of CHD was estimated for both

fasting and postload tHcy concentrations. The odds ratio for fasting tHcy levels in patients as compared to controls was 1.2 [(1.04-1.3), p=0 .008] and 1.1 [(1.0-1.11), p=0.03] for postload tHcy levels. Among patients with elevated fasting tHcy, the odds ratio was 1.9 [(1.3-2.5), p=0.00]. For elevated postload tHcy, the odds ratio was lower but remained significant [odds ratio = 1.2 (1.1-1.39), p= 0.00] (Table III). The observed odds ratio in our study is consistent with those reported by a recent nationally representative study of US adults (NHANES III) relating elevated tHcy levels with MI risk [(21)]. In this study, the odds ratio for hyperhomocysteinemia in blacks and whites was 1.9 (.8-4.2) and 1.8 (1.1-3.1), respectively.

Although the observed odds ratio for CHD was lower among patients without elevated tHcy levels, fasting and postload tHcy levels remained strong predictors for CHD (Table III). The increase in tHcy (postload minus fasting) was not related to CHD risk. In the present study, we illustrated a significant continuous relationship between elevated tHcy levels (fasting and postload) and CHD risk (Fig. 1). The increase in the odds ratio becomes more pronounced in the middle of tHcy distribution. These observations suggest a dose-response relationship between tHcy levels and CHD risk. The estimated odds ratio for CHD per 5 (μmol/L increment of fasting tHcy was 2.1 [(1.2-3.8), p= 0.008]; and 1.3 [(1.0-1.7), p=0.03] for postload levels. Hypertension and hypercholesterolemia were not significantly related to CHD risk among the study population. Only smoking and elevated fasting and postload tHcy levels were strong predictors of CHD. In the presence of other risk factors, the odds ratio for a case with elevated fasting tHcy (12.3 (μmol/L) was increased by 1.15, and 0.05 for elevated postload tHcy levels (33.6 (μmol/L). The odds ratio for a smoker of 20 cigarettes per day with elevated fasting tHcy was (1.0-1.26) (Table IV).

We compared our results with those of other case-control studies relating elevated tHcy levels to the risk of CHD. A meta analysis showed that the estimated relative risk (RR) for coronary artery disease per 5 (μmol/L increase in tHcy levels was 1.6 (1.4-1.7) for men and 1.8 (1.3-1.9) for women [(23)].

Chambers *et al* investigated plasma tHcy levels and its association with CHD risk among 551 male cases (294 Europeans, 257 Indian- Asian) [(24)]. The odds ratio per 5 (μmol/L increment in fasting tHcy was 1.2 (1.0-1.4) for Indian Asians, and 1.3 (1.1-1.6) in Europeans. The corresponding odds ratios for CHD among European subjects that were previously reported by Graham *et al* was 1.3 (1.1-1.6) for men and 1.4 (1.0-2.0) for women [(1)].

The mechanism by which hyperhomocysteinemia is related to CHD risk is not fully understood. One of the clinically proposed mechanisms is that it may affect the cells through formation of injurious oxygen species [(25)]. It may also affect platelet function and the coagulation system [(26)].

As in many developing countries, Jordan experienced trends toward urbanization that is accompanied by changes in life style. According to the Ministry of Agriculture in Jordan, 60 % of the total energy intake is derived from carbohydrates, 20% from fat, and 14% from protein (Ministry of Health 1991). Joubran *et al* indicated that the consumption of vegetables and fruits among Arabs is relatively limited. We believe that this trend may remain because the prices of fruits, vegetables, and fortified cereals are high. In our study population, none of the subjects used vitamin supplementations or was physically active.

Many studies have shown that hyperhomocysteinemia can be lowered by folic acid administration and additional improvement has been achieved when vitamin B_{12} was added. Information on both dietary intake and biochemical status of B vitamins among Jordanians is not available. Therefore, efforts should be made to satisfy the needs in this research area. In a recent prospective, double-blinded, randomized trial that involved 205 patients who had successful coronary angioplasty, Schnyder *et al* examined the effect of Hcy lowering with a combination of folate, vitamin B_6, and vitamin B_{12} on restenosis. The subjects were divided into 2 groups to which they were randomly assigned to receive either folate treatment (1 mg folic acid, 400μg vitamin B_{12}, 10 mg pyridoxine) or a placebo. The results showed significant reductions in plasma Hcy levels and in the rate of restenosis after coronary angioplasty ($P < 0.00$). The authors suggested that lowering Hcy levels by folic acid supplementation should be considered in the treatment of patients undergoing coronary angioplasty [(27)]. Due to the observed similarities with regards to the prevalence of risk factors for CHD among our study population, it is suggested that screening for controls should be more intensive and rigorous. Finally, based on our observations, we suggest that routine screening for hyperhomocysteinemia should be considered particularly among patients with high-risk profile.

Conclusion

The present study illustrates for the first time that hyperhomocysteinemia is significantly and independently associated with coronary heart disease in our Jordanian sample.

Limitations of the Study

The present study had some limitations. Homocysteine levels are inversely related to B vitamins including folate, cobalamine, and vitamin B_6. Information on dietary intakes and serum levels of these vitamins among the study population was not available. Hence, the extent of the relationship between tHcy levels and B vitamins as risk factors for CHD could not be fully explored. Second, the number of subjects that were examined as compared to those in other case-control studies was relatively small. Methionine has an unpleasant taste and methionine loading is difficult to apply, therefore, recruitment of subjects was not feasible. Matching the cases with subjects from the personnel was relatively difficult due to demographic variations between both groups. Subjects from the personnel or their relatives were younger and mostly males, which made the matching more complicated. Therefore, recruitment of controls was performed without matching with cases of CHD. However, in spite of the relatively small sample size, the strength of the association between tHcy levels and CHD risk that were obtained by many statistical models contributes to the strength of these findings. Finally, the number of controls was lower than cases.

Table I. Demographic, clinical, laboratory characteristics among cases and controls.

Variables	Patients (n=45)	Controls (n=35)	P. Value
Mean age*, years	46.7 ± 8.02	47.6 ± 7.7	0.6
Males (%)	35 (77.7)	25 (71.4)	0.6
Females (%)	10 (22.2)	10 (28.5)	0.6
Body Mass Index*, Kg/m^2	29.6 ± 7.7	27.4 ± 4.6	0.1
Systolic Blood Pressure, mm Hg*	112 ± 16.46	118 ± 16.5	0.06
Diastolic Blood Pressure, mm Hg*	71.33 ± 11	74.43 ± 8.64	0.1
No. (%) with Hypertension †	13 (28.8)	11 (31.4)	0.81
Mean Cholesterol Levels, mg/dL*	191.13 ± 47.0	181± 45.0	0.34
No. (%) of Current Smokers	27 (60)	16 (45.7)	0.08
Mean Cigarettes/day	14.93 ± 13.6	10.6 ± 12.3	0.14
Mean Fasting Homocysteine, µmol/L*	14.12 ± 7.39	10.0 ± 3.8	0.003
Mean Postload Homocysteine, µmol/L*	32.86 ± 12.37	27.6 ± 7.12	0.02
Total Homocysteine Increase, µmol/L‡	18.7 ± 8.6	17.7 ± 5.5	0.52

* Values are expressed as means ± SD. † Subjects using antihypertensive drugs. ‡ Postloading minus fasting total homocysteine.

Table II. Fasting homocysteine concentrations by CHD risk factors for patients and controls.

	Homocysteine Concentrations* (µmol/L)			
Variable	**No. (%)**	**Patients**	**No. (%)**	**Controls**
Smoking Habit				
Nonsmokers†	5 (11.1)	19.11^a ± 8.8	13 (37.1)	10.45^b ± 4.3
Exsmokers	13 (28.8)	11.76^a ± 7.8	6 (17.1)	12.8^a ± 3.9
Smokers	27 (60)	14.65^a ± 6.97	16 (45.7)	8.4^a ± 2.6
Cholesterol (mg/dL)				
< 200†	30 (66.6)	15.08^a ± 7.8	22 (62.8)	9.89^b ± 3.9
≥ 200	17 (37.7)	12.27^a ± 5.6	11 (31.4)	9.5^a ± 3.8
Hypertension	13(28.8)	11.55^a ± 5.9	11 (31.4)	9.8^a ± 3.7
No Hypertension†	32 (71.1)	15.16^a ± 7.7	24 (68.5)	10.02^b ± 3.9
Gender				
Males	35 (77.7)	13.9^a ± 6.5	25 (71.4)	10.15^a ± 3.7
Females	10 (22.2)	14.56^a ± 10.5	10 (28.5)	10.05^a ± 4.4
Age				
29-39 years	9 (20)	14.15^a ± 9.3	5 (14.2)	11.6^a ± 3.8
40-50 years	21 (46.6)	12.7^a ± 5.1	17 (48.5)	9.5^a ± 4.2
51-60 years	15 (33.3)	16.0^a ± 8.8	13 (37.1)	9.8^a ± 3.2

*Values are presented as means ± SD.

†Means in the rows with unlike superscripts (a,b) are significantly different ($p \leq 0.05$).

Table III. Odds ratio for CHD in subjects with and without elevated homocysteine*

Variable	Odds ratio (95% CI)	P. Value
Elevated Homocysteine †		
Fasting	1.9 (1.3-2.5)	0.000
Postload	1.2 (1.1-1.39)	0.000
No Elevated Homocysteine †		
Fasting	0.8 (0.72-0.95)	0.01
Postload	0.9 (0.84-0.97)	0.009

*Patients with tHcy levels above and below the 75th percentile of the controls distribution.

† Reference group: Controls below the 75th percentile of controls distribution.

Table IV. Odds ratio for elevated fasting and postload homocysteine with other risk factors (Adjusted for Age and Gender)

Variable	Odds ratio (95% CI)	P. Value
Elevated Homocysteine*		
Fasting	3.0 (1.5-5.9)	0.001
Hypercholesterolemia†	1.4 (0.2-8.4)	0.6
Hypertension‡	0.8 (0.11-5.2)	0.8
Smoking (> 20 Cigarettes/d)§	1.1 (1.03-1.26)	0.007
Elevated Homocysteine*		
Postload	1.3 (0.17-3.6)	0.0002
Hypercholesterolemia†	0.9 (0.17-3.6)	0.7
Hypertension‡	0.6 (0.09-3.4)	0.5
Smoking (> 20 Cigarettes/d) §	1.0 (1.0-1.16)	0.01

*Patients with tHcy levels above the 75th percentile of the controls distribution as compared to controls below the 75th percentile of the controls distribution. †Subjects with cholesterol levels > 200 mg/dL. ‡Subjects using antihypertensive drugs.
§Smokers of 20 cigarettes per day or greater as compared to nonsmokers.

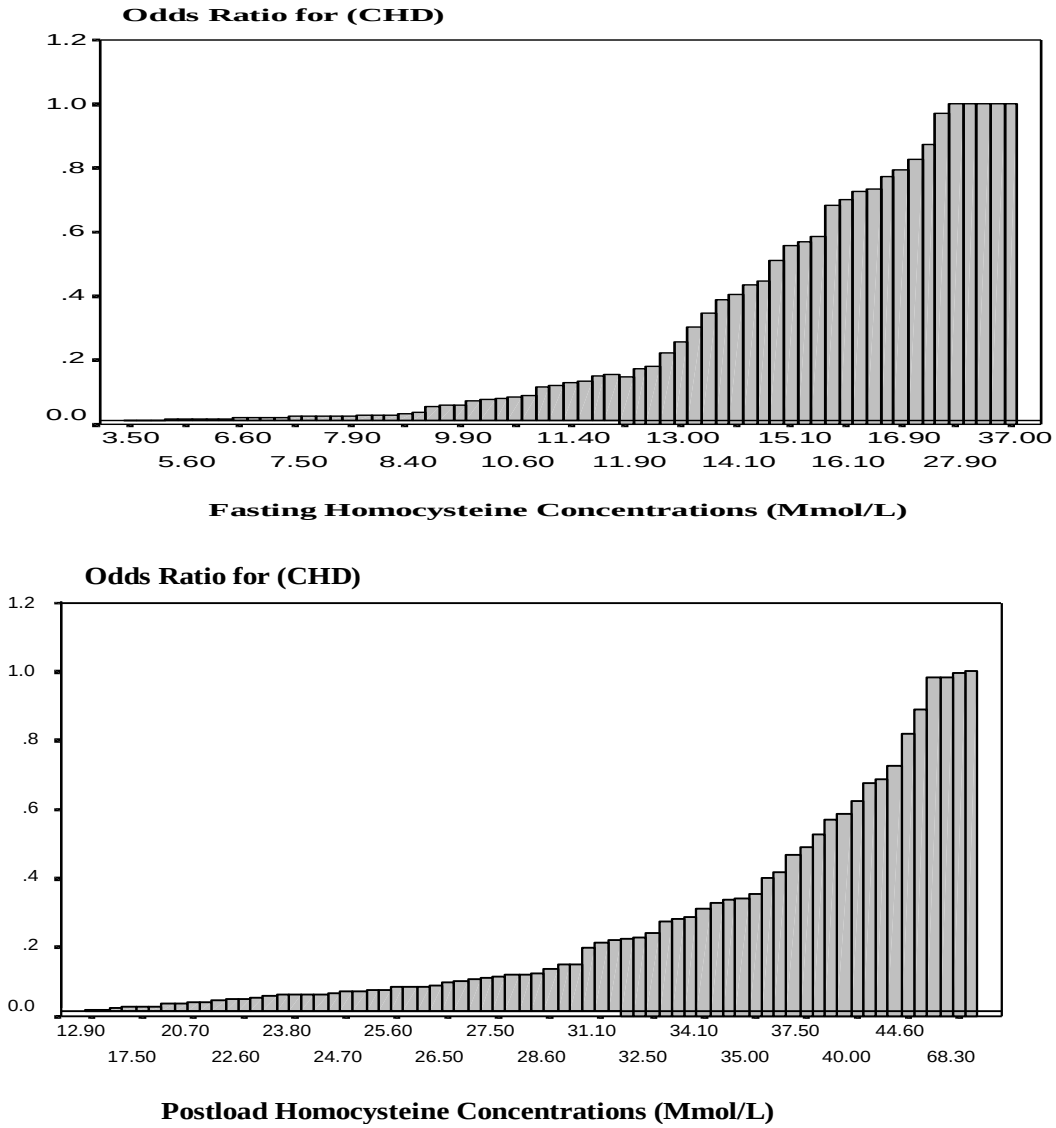

Fig. 1. Association Between Fasting and Postload Elevated Homocysteine Levels and Relative Risk for CHD*
* Mean predicted odd ratios and actual tHcy levels.

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