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The Relationship Between Plasma Homocysteine Levels and Early Coronary Artery Disease

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Abstract: Recent studies show that hyperhomocysteinemia is an independent risk factor for atherosclerosis, especially coronary artery disease. The variations in plasma homocysteine levels in different populations have made it difficult to establish a threshold level for plasma homocysteine. The aim of this study, was to determine a plasma homocysteine level as a threshold, above, which there is a risk for the development of early coronary artery disease. This study was conducted, as a case-control study on 294 patients under the age of 45, who were hospitalized for coronary artery angiography in 2 academic centers in Tehran, with regard to the known risk factors for coronary artery disease and measures of plasma homocysteine levels. When the study exclusion criteria were applied, 243 people remained in the study. Subsequently, 102 people out of the remaining population (42.9%) had angiographically-proven coronary artery disease. Following the verification of other CAD risk factors in both groups and exclusion of their impacts with statistical methods, the influence of plasma homocysteine levels was studied. The risk of early coronary artery disease increased significantly at a plasma level of 14.4 μ mole L^{-1} (OR = 1.99; p = 0.036). At higher homocysteine levels, the chance of early coronary artery disease increased proportionately in a way that a three-fold increase was observed (noted) at levels higher than 23.5 μ mole L^{-1}.

Key words: Homocysteine, atherosclerosis, early coronary artery disease

INTRODUCTION

Homocysteine is an amino acid containing a sulfhydryl group, which is produced by demethylation of methionin, an essential amino acid that is found in food (Yong *et al*., 2002). The reverse process or the conversion of homocysteine to methionin is mediated by methionin synthase, which requires vitamin B12 as a cofactor. In addition, the enzyme CBS (Cystathionine B Synthase) catalyzes the conversion of homocysteine into cystathionine. In all these reactions, vitamin B12 and folic acid serve as coenzyme and methyl provider in the body, respectively (Jee *et al*., 2002). For this reason, folic acid and vitamin B12 deficiency may reduce the activity of mTHFR and CBS and lead to the impairment of methionin synthesis and therefore, homocysteine accumulation (Finkelestein *et al*., 1998).

In contrast to severe cases of hyperhomocysteinemia (THCY >100 μ mole L^{-1}) (Mangoni and Jackson, 2002), which is often seen in inherited defects of methionine metabolism, mild to moderate hyprhomocysteinemia (THCY>15 μ mole L^{-1}) (Mangoni and Jackson, 2002) is prevalent in the general population and is often caused by a deficiency of nutritional factors, such as vitamin B12 and folic acid (Lucock *et al*., 1999; Bree *et al*., 2001; Selhub *et al*., 1993).

Increased plasma homocysteine may also happen as a result of mTHFR- encoding gene polymorphism (Jee *et al*., 2002), intake of folate antagonists like carbamazepine and methotrexate and finally, disorders in homocysteine metabolism due to hypothyroidism or renal failure (Malinow *et al*., 1999; Hankey and Eikelboom, 2000).

In addition to various known risk factors like diabetes, hypertension, hypercholesterolemia, cigarette smoking, etc (Braunwald *et al*., 2001), which predispose to and aggravate atherosclerosis, recent studies demonstrate that increased plasma homocysteine as an independent risk factor has a close association with the aggravation of atherosclerosis and coronary artery disease (Genest *et al*., 1990; Virdis *et al*., 2002). However, fewer studies have been conducted on the role of homocysteine in early onset of coronary artery disease (Mansoor *et al*., 1997; Pinto *et al*., 2001) and this issue still demands deeper attention. In addition to atherosclerosis and vascular diseases, especially coronary artery disease,

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homocysteine and its increased concentrations may be associated with various neurological problems, like inborn defects of neural tube and Alzheimer (Daly *et al*., 1995; Lindenbaum *et al*., 1988). It has also been proposed to have the potential to induce carcinogenicity become carcinogenic (Houlston *et al*., 2001).

Despite various studies, which all imply the risk of elevated levels of homocysteine, no precise and standard definition has been presented for hyperhomocysteinemia. Although some studies claimed to have recognized the concentrations in control population as standard levels (Clark *et al*., 1991), these studies only relied on a wide range-reference presented for homocysteine (Mangoni and Jackson, 2002). Various studies indicate the effect of race, ethnicity, food habits and lifestyle on plasma homocysteine levels (Pancharuniti *et al*., 1994) Even though there are many reports about plasma homocysteine levels in some populations (Manisha *et al*., 2003), few reports are available about plasma homocysteine levels in Iranians (Golbahar *et al*., 2003; Fakharzadeh *et al*., 2006).

In addition to measurement of plasma homocysteine levels in both patients and controls, this study evaluates the risk of CAD due to different levels of plasma homocysteine and, aims to find a homocysteine threshold level, above, which there is a risk for early coronary artery disease.

MATERIALS AND METHODS

Two hundred and ninty four young adults (225 men and 69 women) in 2 case and control groups (118 patients and 156 healthy persons, respectively) were enrolled for/into this case-control study. The study population was selected from among persons who had been hospitalized for coronary artery angiography in 2 academic centers in Tehran and had the qualifications for participation in the study, the most prominent of which was being under 45 years old. The required data were obtained from two references: Part of the data was obtained during interviews upon completion of a fixed questionnaire including all personal information and medical history, which was arranged based on the standard Rose questionnaire for anginal pain, the known risk factors, interfering factors and confounders. Besides, laboratory tests such as blood sugar, homocysteine, triglyceride, cholesterol, etc were conducted. It is noteworthy that the exclusion criteria comprised thyroidal problems such as hypothyroidism, chronic renal disease or renal failure and finally, recent consumption of vitamin B12, folic acid and the other drugs affecting homocysteine levels. Accordingly, 51 people from among the study population (294: 32 males, 19 females) were excluded.

Laboratory tests: Plasma homocysteine was measured using HPLC (High Performance Liquid Chromatography) and Colorimeter was applied for the other measurement.

Data analysis: After the required statistical society had been created and the necessary raw materials had been collected, they were statistically analyzed with the statistical softwares, SAS and SPSS.

Chi-square was used for the evaluation of qualitative variables. For quantitative variables, t-Test and variance analysis were used. In addition, Logistic Regression test was applied for a more precise evaluation. AP. value of <0.05 was considered as meaningful.

RESULTS

When the study exclusion criteria were applied, 243 subjects remained in the study: 193 males (79.5%) and 50 females (20.5%). Notably, all people in this population were under the age of 45. Coronary artery angiography revealed that 102 people (42.9%) had early coronary artery disease: 89 of them (87.3%) were men and 13 (12.7%) were women. In addition, 136 people (57.1%), who had no significant angiographically-proven coronary artery involvement, were considered as controls. The average age range of the subjects was 40.0±4.3 years (40.2±4.1 in men and 39.1±4.7 years in women). The mean plasma homocysteine level in this population was 16.67±1.01 μ mole L^{-1}, which was significantly higher in men (17.95±1.23 μ mole L^{-1}) than in women (11.55±0.90 μ mole L^{-1}), p = 0.011) (Table 1).

In addition, studying this population under the category of predetermined percentiles of plasma homocysteine concentrations showed that the frequency of early coronary artery disease in people in the upper percentile levels is significantly higher than lower percentiles (p = 0.003) (Fig. 1).

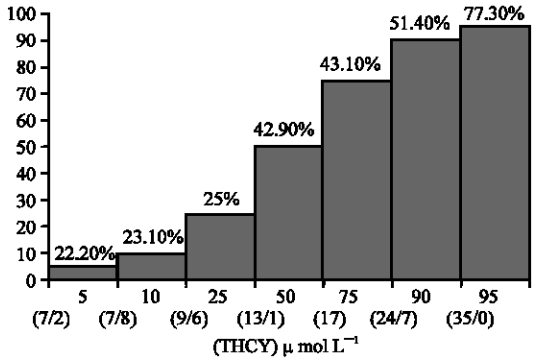

Fig. 1: Frequency of early coronary artery disease in different percentiles of plasma homocysteine concentrations

Table 1: Concentration of plasma homocysteine (μ mole L^{-1}) in case and control groups

	Number	Plasma homocysteine concentration	Plasma homocysteine percentile levels 5	10	25	50	75	90	95
Men	193	17.95±1.23 μ mole L^{-1}	7.40	8.1	10.7	13.9	18.5	27.2	37.6
Women	50	11.55±090 μ mole L^{-1}	6.00	6.7	8.3	10.1	13.4	17.2	18.6
Total	243	16.67±1.01 μ mole L^{-1}	7.20	7.8	9.6	13.1	17.0	24.7	35.0

Table 2: Risk of early coronary artery disease correlated with different plasma homocysteine levels in the study population

THCY (μ mole L^{-1})	Odds ratio (OD)	Confidence interval 95% (CI)	p-value
13	1.85	0.95-3.600	0.0700
14	1.86	0.98-3.570	0.0580
14.4	1.99	1.04-3.810	0.0360
15	2.40	1.23-4.700	0.0100
16	2.29	1.14-4.600	0.0190
17	2.99	1.42-6.290	0.0040
18.7*	4.89	2.10-11.38	<0.0001
22.5**	5.16	1.68-15.86	0.0040

* and **are 90 and 95th percentiles of plasma homocysteine in control group, respectively

Table 3: Risk of early coronary artery disease correlated with different plasma homocysteine levels in men

THCY (μ mole L^{-1})	Odds Ratio (OR)	Confidence Interval 95% (CI)	p-value
14.4	2.19	1.09-4.41	0.0280
15	2.54	1.23-5.22	0.0110
16	2.25	1.08-4.71	0.0310
17	2.98	1.34-6.61	0.0070
18.7*	5.27	2.11-3.17	<0.0001
23.5**	7.24	2.02-25.9	0.0020

* and **are 90 and 95th percentiles of plasma homocysteine in control group, respectively

Comparison of cases and controls showed that mean plasma homocysteine concentrations in patients (19.34±1.76 μ mole L^{-1}) is significantly higher than controls (13.96±0.97 μ mole L^{-1}), p = 0.005. Nevertheless, there was a gender-specific difference: Although, there was a similar correlation in male patients (20.3±1.96 μ mole L^{-1}) compared to male controls (14.9±1.25 μ mole L^{-1}), no significant difference was observed between female patients and controls (11.8±1.32 and 11.5±1.12 μ mole L^{-1}, respectively; p = 0.87).

In order to assess the risk of different homocysteine plasma levels and evaluate its effect on early-onset coronary artery disease, while verifying the prevalence of other known risk factors for coronary artery disease such as a history of diabetes, hypertension, hyperlipidemia, or disorder in lipid and lipoprotein levels, positive family history and cigarette smoking, their effect was excluded with the logistic regression test. Subsequently, different plasma homocysteine levels were set as standard in increasing order and logistic regression test was conducted for each (Table 2 and 3).

In this study, a high risk of early coronary artery disease was observed in patients who had plasma homocysteine concentrations over 14.4 μ mole L^{-1} for example at this level (14.4 μ mole L^{-1}) OD was 1.99 (CI95%:1.04-3.81, P: 0.036) (Table 2). This table depicts the risk of early involvement of coronary arteries in different plasma homocysteine levels (Table 2).

Logistic regression test for each sex led to a very similar result in men. Increased chance of early coronary artery involvement due to high levels of plasma homocysteine was not observed at any level in women.

Although, these tests lack any evidence concerning the effects of homocysteine and its increased concentration on early coronary artery disease in women, the frequency of early coronary artery in women was clearly greater at concentrations over 15 μ mole L^{-1} in contrast to lower concentrations (42.9 and 20.5%, respectively).

Variance analysis, which was used to study the vascular involvement pattern and its association with plasma homocysteine concentration, did not reveal any clear association between the number of involved vessels and increased plasma homocysteine concentration.

Age has been suggested as a major factor affecting plasma homocysteine level. However, this study did not demonstrate any significant difference in plasma homocysteine levels in people under or over 35 years old.

DISCUSSION

Although, a number of recent studies as Moleerergpoom and Guo studiesemphasize the role of high plasma homocysteine levels in the increased risk of coronary artery disease (Moleerergpoom *et al*., 2004; Guo *et al*., 2004) or Lolin and Pinto studies about its early onset (Mansoor *et al*., 1997; Genest *et al*., 1990), no standard, precise definition has been presented for hyperhomocysteinemia up to the present time. Pancharuniti and Wolfgang explained that this may be due to the diversity of plasma homocysteine levels in different societies under the influence of factor such as race, ethnicity (Pancharuniti *et al*., 1994), lifestyle, or even food habits (Herrmann *et al*., 2001). For example, while plasma homocysteine level is as low as 6 μ mole L^{-1} in Japan, it has been reported at 13 μ mole L^{-1} in South Africa. Dr. Alfthan and his colleagues evaluated plasma homocysteine levels in thirteen different countries, in a report that was published in 1997 (Alfthan *et al*., 1997). The plasma levels varied from 7.1 μ mole L^{-1} in Germany to 10.7 μ mole L^{-1} in Finland. The highest mortality rate

due to cardiovascular diseases was reported in Finland and Northern Ireland, which had the highest mean concentration of plasma homocysteine. The reason for this difference is not clearly known, but it may be due to the effect of geographical factors on agricultural products and the food that is processed from them, especially the amount of folic acid in foods in these relatively similar societies. Unfortunately, few studies have been conducted on this issue in Iran and the available data are very limited.

Golbahar *et al.* (2003) conducted a study on plasma homocysteine levels in Department of Biochemistry of Shiraz University of Medical Sciences (Fakhrzadeh *et al.*, 2006). It was reported that the mean plasma homocysteine level is 7.3 μ mole L^{-1} in men and 6.3 μ mole L^{-1} in women. In addition to sex, old age has also been reported as a factor that is effective on plasma homocysteine level. Similarly, Fakhrzadeh *et al.* (2006) conducted a study in Endocrine and Metabolism Research Center of Tehran University of Medical Science (Fakhrzadeh *et al.*, 2006). In this study, mean plasma homocysteine level was 19.02±1.46 μ mole L^{-1} in men and 14.05±1.45 μ mole L^{-1} in women. Male sex, increasing age and folate and vitamin B12 deficiency were all suggested as contributing factors.

In our study, mean plasma homocysteine level in controls was 14.9±1.45 μ mole L^{-1} in males and 11.5±1.12 μ mole L^{-1} in females. Our study resembles Dr. Fakhrzadeh's study in that high plasma homocysteine concentration was relatively high in the studied populations. In this study, logistic regression was conducted for different plasma homocysteine levels, leading to the conclusion that 14.4 μ mole L^{-1} could be considered as a threshold level for plasma homocysteine ($p = 0.036$; OR = 1.99; 95% CI: 1.04-3.81), above which there is a risk of early CAD. It is noteworthy, that as plasma homocysteine concentrations exceed this level, the probability of early coronary involvement increases, so that at concentrations higher than 23.5 μ mole L^{-1} (95th percentile of plasma homocysteine in control group), it becomes double to triple the amount (Table 2 and 3).

It must be noted that this relationship only existed in men. Women did not show any apparent correlation between high plasma homocysteine concentrations and early coronary artery involvement, statistically. Nevertheless, we may not reach a definite result about the effect of homocysteine in women, since the lack of a significant relationship may be due to the small number of women and the scattered distribution of plasma homocysteine concentrations in this population. Remarkably, the frequency of early coronary artery disease in women with plasma homocysteine concentrations over 15μ mole L^{-1} (42.9%) is approximately twice that observed in women with plasma homocysteine concentrations under 15 μ mole L^{-1} (20.5%).

No previous study in Iran had determined a threshold level for plasma homocysteine. The number of studies conducted in other populations is also limited.

Laraqui *et al.* (2002) conducted a study, which assessed homocysteine as a risk factor for coronary artery disease and suggested a plasma concentration of 15 μ mole L^{-1} as a threshold, above which there is a risk for CAD. People who had higher plasma homocysteine concentration had a greater risk of coronary artery disease ($p<0.001$; CI: 3.66-6.66; RR = 5.16).

Skibinska *et al.* (2004) conducted another study, which agreed on 11.21μ mole L^{-1} as the basis for evaluation and suggested that levels exceeding this basis may advance coronary artery disease, whereas the study conducted by Graham *et al.* (1997) suggested plasma levels higher than 14 μ mole L^{-1} as a risk factor for coronary artery disease.

In addition, some studies have evaluated the risk and the incidence as well as progression of CAD (Arnesen *et al.*, 1995) or mortality due to vascular complications (Hoogeveen *et al.*, 2000) resulting from increased plasma concentrations and have suggested it as a major contributing factor. Despite the trace differences between our study and other studies, which may be due to the variable range of homocysteine concentrations in different populations, similar results are deduced from both of them. Thus, we can set the range of 14-15 μ mole L^{-1} (14.4 μ mole L^{-1}) as a threshold for early coronary heart disease and recommend people to alter their lifestyle and even start medical treatment.

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