Original Article

Homocysteine level in CAD patients of Iranian population

M Ghaedi[1], AM Haji Zeinali[1], MA Boroumand[1], SH Abbasi[1,2]

1.Tehran Heart Center, Medical Sciences / University of Tehran, 2.National Iranian Oil Company (NIOC)Health Organizations, Tehran, Iran

Background: Coronary artery disease (CAD) is emerging as a major public health concern in most developing countries. Several studies showed that elevated plasma homocysteine level is a risk factor for CAD. The present study was conducted to determine the level and reference intervals of plasma homocysteine in CAD patients and normal individuals in a selected Iranian population.
Patients and Method: Total plasma homocysteine concentration in 100 patients with CAD and 100 normal controls were measured using homocysteine measuring kit by Elisa method.
Result: Plasma homocysteine concentration was significantly raised in Iranian CAD cases compared to the normal control (15.56 versus 11.51, $p<0.05$). Reference intervals were 7.8-16.1 μmol/L.
Conclusion: An increase in plasma homocysteine concentration can be considered a major risk factor for CAD in selected Iranian population.

Key Words: Homocysteine, CAD, Folate, Reference intervals

Introduction

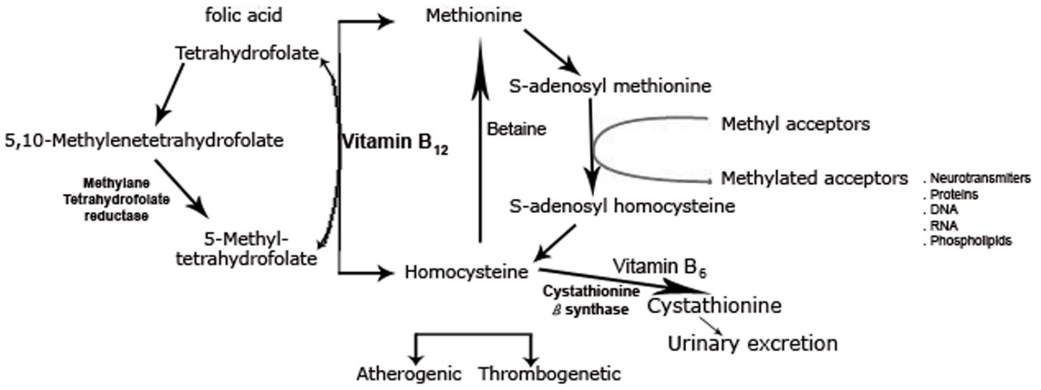
Coronary artery disease (CAD) is the major cause of death in industrial nations. Despite advances in our understanding of cardiovascular disease, traditional risk factors such as hypertension, smoking, diabetes mellitus and dislipidemia do not accurately predict cardiovascular events[1,2]. Homocysteine is an emerging new risk factor for coronary artery disease. Numerous clinical studies have shown that total homocysteine is a risk factor for coronary artery disease and stroke in humans and predicts mortality independently of traditional risk factors in patients with CAD[3].

Homocysteine is a thiol containing amino acid produced from methionine metabolism that dose not participate in synthesis of protein[4]. Mild to moderate hyperhomocysteinemia is a well established independent risk factor for coronary, cerebral and peripheral atherosclerotic diseases and venous thrombosis[5-9].

Homocysteine is generated in a cycle through S. adenosyl methionine (SAM) and S. adenosyl homocysteine and is involved in two main metabolic pathways; transsulforation in which homocysteine is converted to cysteine by B6-dependent cysthathionine B synthase and remethylation of homocysteine back to methionine which is carried out by vitamin B12-dependent methionine synthase and bataine-homocysteine methyl transferase (BHMT).The

Correspondence:
Seyed Hesameddin Abbasi MD,
Research Department, Tehran Heart Center, North Kargar Street, Tehran, Iran.
Tel: +98 9121575226 Fax: +98 21 88029256
E-mail: abbasi@tehranheartcenter.org

Figure 1.Homocysteine metabolic pathways



In the methylation pathway, homocysteine acquires a methyl group either from betaine (a reaction that occurs mainly in the liver) or from 5-methyltetrahydrofolate (a reaction that occurs in all tissues and is vitamin B12-dependent). In the transsulfuration pathway, homocysteine is metabolised to cystathionine in a reaction catalysed by cystathionine β synthase and requiring vitamin B6.

folate cycle generates 5- methylylenetetrahydrofolate for remethylation of homocysteine back to methionine[10], (Fig. 1).

Until recently it was believed that the normal range for homocysteine was 5 to 15 μmol/L . It is now widely accepted that upper range of normal may be 10 to 12 μmol/L for middle-aged adults and values exceeding this range considered to be a risk factor for cardiovascular disease. Patients with coronary artery disease and other cardiovascular diseases usually have mild hyperhomocysteinemia (>12 to 25 μmol/L) with an incidence of 30 to 50 percent[11].

Plasma homocysteine concentration are determined by both genetic and nutritional factor deficiencies of folate, vitamin B12 or B6 which can lead to impaired homocysteine metabolism and hyperhomocysteinemia[11-13]. In addition , mutations in the genes coding for methylenetetrahydrofolate reductase (MTHFR), methionine synthase and cysthathionine B synthase may also produce hyperhomocysteinemia. Smoking, excessive coffee consumption and lack of exercise are also associated with elevation in homocysteine[14,15]. Because homocysteine has a thiol , it can undergo autooxidation and oxidation with other thiols. The resulting reactive oxygen species, hydrogen peroxide and superoxide anion radical , generate oxidative stress and induce vascular dysfunction[16,17]. Recent evidence suggests that homocysteine may limit the bioavailability of nitric oxide resulting in the impairment of folate-mediated vasodilation. The limited bioavailability of nitric oxide could be due to nitrosothiol formation with homocysteine. Homocysteine may also target specific proteins and impair their activity and function through disulfide bond formatio[18]. The decreased binding of tissue plasminogen

activator to homocysteine-modified annexin may explain, in part, the procoagulant activity of homocysteine. Finally, homocysteine appears to induce the expression and secretion of chemokines such as monocyte chemoattractant protein 1 (MCP1) and interlukin 8 (IL-8) in vascular endothelial cells. Production of these chemokines by estimulated endothelial cells would attract monocytes and neutrophils to sites of vascular injury where they could localize in the intimal space, transform into macrophages, engulf oxidized LDL, and become foam cells. Foam cells are a source of reactive oxygen species which can play a role in other sequences of events that promote atherosclerosis[19,20].

A wealth of epidemiological evidence from >100 prospective cohort, cross sectional and case control studies have confined the relationship between homocysteine concentration and vascular disease[21-23]. However, there is no data available from Iranian patients. We speculated that there was a possible link between homocysteine concentration and CAD .The aim of the present study was to determine the reference intervals for plasma homocysteine and explore an association between plasma homocysteine concentration and CAD in selected Iranian population.

Patients and Methods

This study was performed on 100 adult cases of angiographically documented CAD in Tehran Heart Center and 100 normal controls from the general population in Tehran. The patients consisted of 61 males and 39 females with an average age of 56.9 years.

The data obtained through interviewing all the participants, comprised detailed clinical, family and medication histories, as well as dietary and smoking habits, socioeconomic status, , diabetes mellitus and hypertension.

Normal control group (72 males and 28 females) were healthy volunteers without any clinical disorders and no history of CAD, hypertension, Smoking diabetes mellitus or cardiovascular disease. Their age averaged 45 years, and had no clinical or investigative evidence of vascular, renal, hepatic or metabolic diseases.

Informed consent was obtained from each patient and healthy subject according to the guidelines of our ethics committee. Demographic data of study population are presented in Table 1.

Homocysteine

Blood samples collected from cases and controls in the sitting position. Total homocysteine was measured in plasma because the use of anticoagulant allowed immediate sample processing. Serum, even if optimally prepared, yielded slightly higher values than plasma.

Venous blood sample were taken from fasting individuals into EDTA vials. The samples were immediately ice packed and centrifuged within 30 min to avoid release of homocysteine from red blood cells. Plasma samples were then refrigerated and stored at -20 C until used. Total plasma homocysteine concentration of patients and normal controls were determined by ELISA, using homocysteine measuring kit.

Statistical analysis

Result was presented as mean and standard deviation. Data were analyzed with SPSS version 10.0, plasma homocysteine was taken as a continuous variable to allow for risk

Table 1. Demographic data of study population

Study population	Sex (M : F)	Mean age(rang)
CAD patient	61:39	57 (32-89)
Normal control	72:28	45 (32-55)
Total	133:67	48 (32-89)

Table2. Distribution of plasma homocystiene concentration (μ/mol) among CAD cases and controls group

	<5	5-15	15-30	>30
Cases	2	53	42	3
Control	2	86	10	2

analysis. Differences between groups were evaluated by using student t-test. P value <0.05 were considered statistically significant.

Result

Total plasma homocysteine concentrations were measured in 100 CAD cases and 100 normal controls to determine the reference limits of plasma homocysteine for selected Iranian population and its possible relationship to CAD. The mean homocysteine in cases and controls were 15.56±6.77 and 11.51±4.63 respectively (P value<0.001). The mean level of plasma homocysteine in 100 normal individuals was 11.51 and 95 percentile or reference intervals ranged from7.8 to 16.1 μmol/L. As demonstrated in Table 2, mild hyperhomocysteinemia (homocysteine >16.1 μmol/L) were more common in the CAD patients (42%) than in control group (10%). The proportion of subjects with moderate hyperhomocysteinemia was substantially higher among patients with CAD than controls.

Discussion

In the past decade, total plasma homocysteine emerged as a novel risk factor for cardiovascular disease[24] including CAD which is the major cause of death in industrial nations. The involvement of homocysteine in vascular disease may, at least in part, be due to its metabolic conversion to HcyT, thereby modifying protein lysine residues, and causing protein and cell damage[25,26]. It can also undergo autooxidation and oxidation by other thiols. The resulting reactive oxygen species generate oxidative stress and caused endothelial cell damage, increased platelets aggregation, oxidation of LDL and proliferation of vascular smooth muscle cell. Such events promotes atherosclerosis[16-18].

Studies have estimated that a 5 μmol/l homocysteine increased CAD risk as much as cholesterol increase of 20 mg/dl. On the other hand, a prolonged decrease of homocysteine by 3–4 μmol/l was associated with 30–40% reduction in risk of CAD[1,26].

In our study, we determined the reference intervals for plasma homocysteine and investigated a possible relationship between homocysteine and CAD in our selected population. The homocysteine mean in cases and controls were 15.56±6.77 and 11.51±4.63. Plasma level of homocysteine in 100 normal individuals has mean of 11.51 and 95 percentile were 7.8 to 16.1. Therefore in regard to reference intervals for plasma homocysteine in our selected population, mild hyperhomocysteinemia (homocysteine >16.1 μmol/L) were more common in the CAD patients (42%) than in controls group (10%).

In line with several previous studies, the foregoing data indicated that elevated plasma homocysteine concentrations are likely to be a risk factor for coronary artery disease in selected Iranian patients[27-30].

The first report by Mayer and Jacopson in 1996 showed that homocysteine increased the risk of CAD[21]. This was supported by Refsum H study on the effect of plasma homocysteine concentrations on CAD[31,32]. The effect of plasma homocysteine on cardiovascular disease was discussed in a critical review in 1999[30]. In meta analysis carried out by Boushy and coworkers, showed that 10% of CAD correlated with hyperhomocysteinemia[33]. Another study performed on 5000 CAD patients reported that there was a significant relationship between homocysteine and cardiovascular disease. Elevation in homocysteine level was associated with 0.9-4.5 fold increase in the risk of CVD[34,35].

However until now epidemiological evidence from >100 prospective cohort, cross sectional and case control studies have confined the relationship between homocysteine concentration and vascular and ischemic heart diseases, thrombosis atheriosclerosis and CAD[21,22,31]. In view of the small number of subjects participating in our study, further investigations employing larger sample size are needed to accurately determine reference intervals in selected Iranian population. Furthermore, it is also important to launch large-scale prospective clinical trials in order to establish the role of homocystein as a risk factor for CAD among Iranian population. Depending on the results obtained, the administration of folic acid, vitamin B12 and pyridoxine to lower homocysteine concentrations would then be considered in order to prevent cardiovascular disease. Further aspects to be studied include the roles played by genetic, physiologic and life style factors, diabetes mellitus, hypertension, total levels of triglyceride, HDL, LDL, cholesterol and blood concentrations of B vitamins, particularly folate and cobalamin.

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