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Frequency of Afterload Homocysteinemia in Normal Population of Southern Iran: A Pilot Study

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Abstract: The objectives of the present pilot study were to investigate the effect of an oral methionine load on plasma homocysteine in healthy subjects southern Iran. We studied 50 peoples (10 men, 40 women, median age 27.5, range 20-37) referred to screening center for marriage since different part of southern Iran. Methionine (0.1 g kg^{-1} b.wt.) was immediately administrated orally in 200 mL of orange juice and a second blood was obtained 4 h later. Plasma level of homocysteine was carried out by high performance liquid chromatography and flumetric detection. A homocysteine level above 15 $mmol\ L^{-1}$ was considered high. The mean fasting and afterload homocysteine were 15.28 and 31.29 $\mu mol\ L^{-1}$, respectively. Fasting hyperhomocysteinemia (>15 $\mu mol\ L^{-1}$) was detected in 12% of male and 8% in female which significantly higher in men than women (p<0.0001). Afterload methionine load homocysteine levels (>31 $\mu mol\ L^{-1}$) was detected in 16% of male and 14% in female which higher in men than women. Notably 80% of participants had normal total homocystein concentration ($\leq$15 $\mu mol\ L^{-1}$), but mild and moderate hyperhomocysteinemia was detected in 24% (n = 12). In this study, we find the difference between fasting and methionine afterload mean homocystein levels (p = 0.000), in 8% of those normal homocystein level, methionine afterload homocystein levels became abnormal. In conclusion, based on results, we recommend the methionine afterload homocystein levels in high risk cases with normal fasting level in order to unmissed some cases with normal basal homocystein level.

Key words: Hyperhomocysteinemia, frequency, cardiovascular disease, afterload, methionine load

INTRODUCTION

Elevated plasma total homocysteine is an independent risk factor for cardiovascular disease and a sensitive marker of the inadequate vitamin B_{12} and folate insufficiency. Folate and vitamin B_{12} have had a protective effect on cardiovascular disease (Fakhrzadeh et al., 2006).

Factors such as age, gender, racial and ethnic differences, geographical variations, genetic, dietary and other life style have shown to be determinants of plasma homocysteine concentrations. Total plasma homocysteine levels are higher in men than in women and also in older age. Elevated fasting homocysteine concentrations are associated with lower circulating concentrations and intakes of folate and vitamin B_{12}. From the studies in different populations around the world, it is now believed that metabolism of homocysteine may be race-ethnic dependent (Jamal et al., 2004).

Elevated plasma levels of homocysteine have adverse effects on the cardiovascular system including enhanced oxidation of low-density lipoprotein,

proliferation of smooth muscle cells, increased platelet adhesiveness and endothelial cytotoxicity. The clinical consequences of hyperhomocysteinaemia are an increased risk of atherosclerotic coronary, cerebral and peripheral vascular disease and also deep vein thrombosis and thromboembolism. A clear association between plasma homocysteine concentrations and mortality has been demonstrated in patients with angiographic coronary artery disease (Lewandowski et al., 2003).

Hyperhomocysteinemia, either fasting or after oral methionine loading, appears to be an independent risk factor for coronary heart disease. It remains unclear whether fasting total homocysteine determination alone adequately detects the full spectrum of hyperhomocysteinemic individuals (Bostom et al., 1995). In the general population, mild to moderate elevations in plasma homocysteine (15 to 35 $\mu mol\ L^{-1}$) are common and may be due to inherited enzyme variants and/or a relative deficiency of folate, vitamin B_{12}, or vitamin B_6, which are required for the normal metabolism of homocysteine. Methionine taken orally is converted to homocysteine by demethylation and the effect of an oral load can be used

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as a diagnostic test to identify individuals with enzyme defects who show an exaggerated rise in homocysteine levels (Bostom *et al.*, 1995).

The objectives of the present pilot study was to investigate the effect of an oral methionine load on plasma homocysteine in healthy subjects Southern Iran.

MATERIALS AND METHODS

This is a pilot study that was done in Outpatient clinic affiliated to Shiraz University of Medical Sciences in southern Iran during 2008.

We studied 50 peoples (10 men, 40 women, median age 27.5, range 20-37) referred to screening center for marriage from different parts of Southern Iran.

Exclusion criteria included known Coronary Heart Disease (CHD), systemic illness, serious organ disease, proliferative and endocrine diseases, alcoholism, pregnancy, current use of vitamins or other supplements, anticonvulsant and anticancer therapy. participants underwent a standardized medical history, physical examination and laboratory tests.

This study was approved by medical ethics committee of Shiraz University of Medical Sciences. Informed consent form was taken before the study.

Laboratory analysis: Blood was drawn between 7.30 and 8.30 a.m. after fasting for 12 h into tubes containing EDTA for plasma levels of homocysteine. Methionine (0.1 g kg^{-1} b.wt.) was immediately administrated orally in 200 mL of orange juice and a second blood was obtained 4 h later. In order to measure homocysteine, the blood sample was immediately put in an ice bath and centrifuged at 3000x g for 20 min at 4°C. Plasma then frozen at -80°C until assay could be performed. plasma level of homocysteine was carried out by high performance liquid chromatography (HPLC, Double pump, Model 1525, Water's Co., USA) and flumetric detection (Multi Landa, Model 2475, Water's Co., USA).

The intra-assay coefficient of variation of this test is 2.5% in our hands.

A homocysteine level above 15 mmol L^{-1} was considered high (Sharifkazemi *et al.*, 2006).

Statistical analysis: Statistical analysis was performed using the SPSS package version 11.5 (SPSS Inc, Chicago, II, USA). For comparison we used also Chi-square and fisher exact test. p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The group studies contained 10 (20.4%) male and 40 (79.6%) female 20-37 years old individuals. Table 1

Table 1: The Mean+1standard deviation (SD) and Mean+2SD of fasting and afterload methionine load homocysteine levels in participants

Homocystein level	Mean	Mean+1SD	Mean+2SD
Fasting	15.28	26.56	38.66
Afterload	31.29	47.36	63.43

shows the clinical characteristics and other family risk factors in the study participants.

The mean fasting and afterload homocysteine were 15.28 and 31.29 µmol L^{-1}, respectively.

Fasting hyperhomocysteinemia (>15 µmol L^{-1}) was detected in 12% of male and 8% in female which significantly higher in men than women (p<0.0001).

Afterload methionine load homocysteine levels (>31 µmol L^{-1}) was detected in 16% of male and 14% in female which higher in men than women.

Notably 80%of participants had normal total homocystein concentration (≤15 µmol L^{-1}), but mild and moderate hyperhomocysteinemia was detected in 24% (n = 12).

Distribution of participation according to their fasting and after methionine load homocysteine concentrations are displayed in Table 1.

Comparing the means of fasting and afterload homocysteine is showed statically significant different (p = 0).

This study set out to investigate the effect of methionine loading on plasma homocysteine levels in healthy control. We used the methionine-loading test to reveal additional abnormalities of the homocysteine metabolism.

Previous studies have suggested that postmethionine-load homocysteine in the absence of fasting hyperhomocystinaemia could account for over 40% of all hyperhomocystinaemic persons (Bostom *et al.*, 1995).

Boers (1994), using a methionine-loading test, identified hyperhomocystinaemia in 14 of 50 patients (28%) with premature peripheral and cerebral arterial disease under 50 years of age. Brattström (1996) reported methionine intolerance in 26 of 72 patients (36%) with cerebrovascular disease who were under 55 years of age.

Ozkan *et al.* (2003) reported In three of 21 women and three of 44 men with the methionine-loading test, plasma homocysteine levels were higher than 30 µmol L^{-1}.

Mild hyprerhomocysteinemia is considered as risk factor for venous and arterial thrombosis. There is some evidence in favour of the role of hyprerhomocysteinemia as an inducer of oxidant stress, other possible mechanism include activation of factor V, interference with protein C activation and thromomodulin expression, inhibition of tissue plasminogen activator binding, and some other mechanisms (Francesco *et al.*, 2003).

Total plasma homocystein levels are higher in men than in women and at older ages. Elevated fasting

homocystein concentration in turn, are usually normalized by treatment with folic acid and vitamin B_{12}. Although epidemiologic survey has determined total homocystein concentrations in order to identify the prevalence of hyprerhomocysteinemia, estimation of these cases is complicated by the lack of a standard definition of a high total homocystein concentration (Fakhrzadeh *et al.*, 2006). The reviews provided in discussion whether these are in support of our results.

In present study, we find the difference between fasting and methionine after load mean homocystein levels (p = 0.000), in 8% of those normal homocystein level, methionine afterload homocystein levels became abnormal. In conclusion, based on results, we recommend the methionine after load homocystein levels in high risk cases with normal fasting level in order to unmissed some cases with normal basal homocystein level.

In this pilot study, we don't find any correlation between family history of thrombosis, cardiovascular diseases, diabetes mellitus, smoking, alcoholism and also participation diet regimen with fasting and afterload homocysteine levels.

Because this study was done as pilot study, we suggest the wide epidimiological study for better evaluation of methionine afterload homocystein level in south of Iran.

ACKNOWLEDGMENTS

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