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Plasma total homocysteine levels in a healthy Turkish population sample

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Objective — The objective of this study is to determine the reference values of homocysteine levels from a sample of healthy native Turks, and the relationship of these levels with gender, age and other risk factors.

Methods and results — Plasma homocysteine level was measured in 159 healthy Turkish individuals. Homocysteine levels were determined by the HPLC method and differences between sex and age groupings (20-40 years, 41-60 years, and 61 and older) were compared. Mean homocysteine levels were 8.91 ± 1.41 μmol/l. The median homocysteine level was 8.35 μmol/l (men 8.80, women 7.0). Homocysteine levels significantly increased with age ($r = 0.49$) and higher in men than in women in each age group ($p < 0.05$) (men: 9.51 ± 1.40; women 7.38 ± 1.36; $p < 0.001$). The cut-off point for high homocysteine level is selected to be the value that marks the upper 20% of the control population distribution (12.26 μmol/l). Postmenopausal > 60-year-old women manifested significantly higher increases in total homocysteine concentrations than 20 to 40-year-old premenopausal women. There were no significant correlations between homocysteine and body mass index, glucose, total and lipoprotein lipids, C-reactive protein, creatinine, smoking and alcohol consumption except blood pressure and uric acid.

Conclusions — These data indicate the significance of sex- and age-associated differences of homocysteine levels in native Turkish subjects. Upper reference limits for the plasma total homocysteine concentration increased with age and were higher for men than for women at all ages. Focusing public health initiatives on this issue may reduce the high prevalence of cardiovascular disease in the Turkish population. *(Acta Cardiol 2006; 61(1): 35-42)*

Keywords: *homocysteine – Turkish population – age – gender – increase in vascular disease risk.*

Introduction

Homocysteine (Hcy) is an intermediate formed during the metabolism of methionine, an essential sulphur-containing amino acid supplied from dietary proteins[1,2]. A large number of epidemiological studies has shown an association between mildly to moderately elevated blood concentrations of total Hcy and atherothrombotic vascular disease (including its coronary, cerebral, and peripheral manifestations)[3-6]. Mild hyperhomocysteinaemia is reported to occur in 5-7% of the general population[7,8]; these people being at higher risk of premature coronary artery disease, as well as recurrent arterial and venous thrombosis[9,10]. In a multicentre case-control study in Europe, elevation of total homocysteine (defined as > 80th percentile of controls; 12.0 μmol/l fasting Hcy) appeared to be at least as strong a risk factor for vascular disease in women as in men, even before the menopause[11]. We have shown that high plasma levels of Hcy in Turkish subjects are associated with coronary artery disease (CAD)[12]. We found that Hcy levels were significantly higher in CAD patients compared to age- and sex-matched healthy controls. We have also presented evidence indicating that an increased plasma Hcy level is an independent risk factor for angiographic restenosis after percutaneous transluminal coronary angioplasty (PTCA) and coronary stenting[13].

Despite increased interest in measurement of fasting and post-methionine load (PML) plasma Hcy

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and the growing number of methodologies used among laboratories, only a few studies on method and inter-laboratory variation have been performed. An external quality assessment programme including nine laboratories from the Scandinavian countries showed that CVs for the measurement of Hcy by high performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS) were 6–12%[14]. This programme was continued, and a later report including 28 laboratories with 34 sets of results using HPLC, GC-MS, and immunoassays, showed an overall within-laboratory CV of 7.5%. Only results obtained with HPLC methods showed significant between-laboratory variance[15]. An international study among 14 laboratories on the comparison of tHcy values obtained on plasma samples with and without added homocysteine showed that mean inter- and intralaboratory variations for HPLC, GC-MS, and immunoassays were < 10%[16]. More recently, a multicentre study on two pairs of pooled plasma of normal fasting and PML tHcy concentrations showed that within-laboratory reproducibility, expressed as median CV, ranged from 2.7% to 3.3% fluorescence polarization immunoassay (FPIA), 9.2% to 13.9% (HPLC), and 21.8% to 24.2% (enzyme immunoassay). Between-laboratory variation ranged from 13.9% to 15.6%[17]. Between-method and between-laboratory variations in serum tHcy analysis are not yet satisfactory; certified reference material and standardization of the plasma tHcy assay will be essential to reduce between-laboratory bias.

A growing body of data indicates that Hcy metabolism may be influenced by genetic background, nutrition, state of health, lifestyle[7,8,18,19]. The literature reports data on healthy "control" subjects, but these subjects typically come from homogeneous cultures or ethnic backgrounds or a particular socio-economic status. A comparative study on healthy subjects from different countries has indicated that the total serum Hcy levels vary between populations (G. Alfthan et al., unpublished observation). For clinicians and laboratory scientists, it becomes necessary to develop reference limits for Hcy population standards, according to age, sex, and ethnicity.

Turkey, a developing country, has a high cardiovascular morbidity and mortality, despite relatively low general levels of plasma cholesterol[20,21]. In the 1990s, incidence and mortality of coronary heart disease became increasingly more important among Turkish adults, considering this population's youthful structure and low plasma cholesterol levels. The prevalence of coronary heart disease (CHD) adjusted for age 35-64 years among Turkish adults has been estimated as 5.8% in men and 5% in women[22]. This rapid increase since the 1990s may be an important risk factor in the Turkish population in terms of hyperhomocysteinaemia[23]. However, the reference values of homocysteine levels and prevalence of hyperhomocysteinaemia in the Turkish population was not yet investigated in detail. The objective of this study is to determine the reference values of Hcy levels in our sample of healthy Turkish subjects and to compare them with other studies from different populations.

Methods

This study is performed in a healthy Turkish population (159 subjects; 41 women, 118 men). All parameters were measured in 2002 to 2003. Fasting plasma homocysteine levels were determined, and differences between sex and age groups (20-40 years, 41-60 years, and 61 and older) were compared.

Informed written consent was obtained from all subjects. All subjects received a complete physical examination, pulmonary function testing, abdominal ultrasonography, stress test, electrocardiogram, typical blood chemistry panel. None of the control group subjects had any clinical evidence of ischaemic heart disease, peripheral vascular disease or cerebrovascular disease, and electrocardiograms were judged normal. Exclusion criteria for all control subjects included a history of diabetes, renal, hepatic or thyroid disease, cancer, gastrointestinal disease, alcohol or illegal drug abuse, psychiatric illness, use of drugs or dietary supplementation with vitamins reported to alter or affect Hcy levels.

All blood samples were obtained from the antecubital vein and drawn into vacutainer tubes containing EDTA. Samples were placed on ice and centrifuged for 5 min (cold centrifuge). The plasma was separated immediately and placed in the refrigerator (2-8°C) and studied the same day. All biochemical parameters were determined from this sample. Plasma samples were frozen if homocysteine was not studied in 12 hours. These frozen samples were studied within two days of collection.

Homocysteine assay

A test kit for the analysis of total plasma homocysteine by high performance liquid chromatography (HPLC) was available from Bio-Rad Laboratories (product #195-4075). A Hewlett-Packard 1100 series HPLC system with G1322A solvent degasser, a 1310A Iso pump, G 1313A auto sampler, G 1316A column heater and 1046A fluorescence detector were used. A reversed phase Micro-Guard cartridge, and analytical cartridge column from Bio-Rad (product #195-4076) were used for analysis. Hcy is measured by fluorescence with excitation at 385 nm and emission at 515 nm. The temperature of the reversed-phase column was set at 45°C. Flow rate of the mobile phase was 0.7 ml/min. For analysis, 20 ml of sample was injected"

The coefficient of variation was 4.2 % for a single sample measured repeatedly.

Biochemical analysis

Fasting and post-load blood glucose, total and lipoprotein lipids, uric acid and creatinine were measured enzymatically (Hitachi 912). C-reactive protein was measured by a Beckman array nephelometre.

Statistical evaluations

Statistical analysis was carried out using the SPSS for Windows 8.0. In the presentation of the results, means ± SD are given for variables. Pearson's correlation coefficients were computed to evaluate the linear associations between homocysteine and other parameters. Comparison between groups was made using Student's t-test, chi-square, Fischer's Exact chi-square tests. $P < 0.05$ was accepted as significantly different. The 80th percentile of the control population distribution of homocysteine level was selected as a cut-off for comparative purposes.

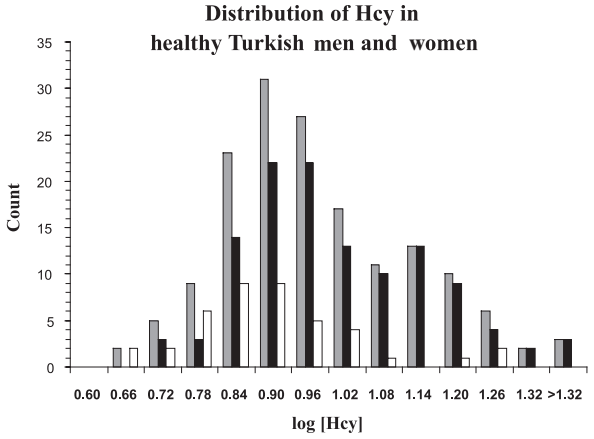

Fig. 1. – Distribution of plasma homocysteine concentrations in healthy Turkish men and women (□ women; ■ men; ▒ all). Logarithmic transformation was used.

Results

Selected characteristics of the study population are shown in table 1. The mean age was 51.85 years with range of 22-88 (men: mean of 52.36, range: 26-86; women 49.78, range: 22-81). The median age was 51 for both sexes combined, 52 for men and 49 for women.

Mean Hcy levels were 8.91 ± 1.41 μmol/l (men: 9.51 ± 1.40; women: 7.38 ± 1.36; $p < 0.001$); values ranged from 4.3 to 22.6 μmol/l. The median was 8.35 μmol/l (men 8.8, women 7). Distribution of plasma Hcy concentrations in healthy Turkish men and women are shown in figure 1. The percentile distribution of plasma Hcy levels are shown in table 2. The cut-off point for high homocysteine level is selected to be the value that marks the upper 20% of the control population distribution (12.26 μmol/l).

Hcy levels increased with age in women and men ($r = 0.49$; men: 0.50; women: 0.47) and the oldest age group had significantly higher homocysteine levels than the other age groups ($p < 0.05$). Hcy levels also differed significantly between men and women in each age group ($p < 0.05$) and were higher in men than in

Table 1. – *Selected characteristics of the study population.*

Variable	All	Men	Women	p*
Mean age	51.85 ± 13.03	52.36 ± 12.68	49.78 ± 14.41	0.847
Mean total homocysteine (μmol/l)	8.91 ± 1.41	9.51 ± 1.40	7.38 ± 1.36	0.001*
BMI (body mass index, kg/m^2) ^{t}	25.5 ± 0.4	25.5 ± 0.5	25.4 ± 0.7	0.896
Current smoker (%) ^{t}	35	36	33	0.914
Alcohol consumption (%) ^{t}	13	18	6	0.127
Mean systolic blood pressure (mm Hg) ^{t}	122.6 ± 1.7	124.4 ± 1.7	119.5 ± 3.4	0.163
Mean diastolic blood pressure (mm Hg) ^{t}	81.4 ± 0.9	83.5 ± 1.0	78.0 ± 1.7	0.004*
Mean fasting blood glucose (mg/dl) ^{t}	96.3 ± 1.8	97.5 ± 2.5	94.3 ± 2.2	0.394
Mean post load blood glucose (mg/dl) ^{t}	116.8 ± 4.7	118.8 ± 6.5	113.3 ± 6.3	0.580
Mean serum cholesterol (mg/dl) ^{t}	227.8 ± 4.3	227.2 ± 4.7	228.7 ± 8.4	0.864
Mean serum triglycerides (mg/dl) ^{t}	146.9 ± 8.2	162.8 ± 11.3	119.1 ± 9.8	0.010*
Mean HDL cholesterol (mg/dl) ^{t}	45.2 ± 1.2	40.8 ± 1.3	53.1 ± 2.0	0.001*
Mean LDL cholesterol (mg/dl) ^{t}	152.8 ± 3.8	154.5 ± 4.3	149.9 ± 7.4	0.560
Mean serum creatinine (mg/dl) ^{t}	1.1 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.011*
C-reactive protein^t	3.6 ± 0.2	3.7 ± 0.2	3.5 ± 0.3	0.714
Mean uric acid^t	5.7 ± 0.1	6.5 ± 0.1	4.4 ± 0.2	0.001*
Coronary heart disease in family (%) ^{t}	35	40	27	0.221
Hypertension (%) ^{t}	8	5	13	0.180

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

^{t}Values were avaible for 117 subjects.

•Tested with Student's t-test for variables (mean and SD are shown) and Pearson's chi-square test for frequencies. * $p < 0.05$

Table 2. – *The percentile distribution of plasma homocysteine levels of the study population.*

Percentile	All	Men	Women
2.5%	4.99	5.57	4.40
5%	5.60	6.09	4.80
10%	6.08	6.67	5.60
20%	6.70	7.10	6.00
50%	8.30	8.80	7.00
80%	12.26	12.96	9.10
90%	14.70	14.93	10.30
95%	17.02	17.29	14.30
97.5%	18.79	20.55	16.20

Table 3. – *Comparison of homocysteine levels between groups.*

Group	Mean Hcy	S.D.	P
Men	9.52	1.40	< 0.05
Women	7.39	1.36	
All 20-40 y	7.56	1.23	0.175
All 41-60 y	8.07	1.35	
All 41-60 y	8.07	1.35	< 0.05
All > 60 y	12.00	1.4	
All 20-40 y	7.56	1.23	< 0.05
All > 60 y	12.00	1.4	
Men 20-40 y	7.90	1.22	< 0.05
Women 20-40 y	6.84	1.21	
Men 41-60 y	8.54	1.32	< 0.05
Women 41-60 y	6.77	1.34	
Men > 60 y	13.00	1.34	< 0.05
Women > 60 y	9.27	1.41	
Women 20-40 y	6.84	1.21	0.901
Women 41-60 y	6.77	1.34	
Women 41-60 y	6.77	1.34	< 0.05
Women > 60 y	9.27	1.41	
Women 20-40 y	6.84	1.21	< 0.05
Women > 60 y	9.27	1.41	
Men 20-40 y	7.90	1.22	< 0.05
Men > 60 y	13.00	1.34	
Men 41-60 y	8.54	1.32	< 0.05
Men > 60 y	13.00	1.34	

women. Postmenopausal > 60-year-old women manifested significantly higher increases in total Hcy concentrations than 20 to 40-year-old premenopausal women. The comparison of Hcy levels between groups is shown in table 3.

There were no significant correlations between plasma total Hcy and body mass index (BMI), current smoking, alcohol consumption, CHD in family, fasting and post-load blood glucose, total and lipoprotein lipids, C-reactive protein, and creatinine in our study group. There were significant correlations between total Hcy and blood pressure and uric acid (table 4) ($p < 0.05$).

Discussion

Hyperhomocysteinaemia as an independent risk factor for cardiovascular disease is thought to be responsible for about 10 percent of total risk[24]. Based on the available evidence, there is an increasing call for the diagnosis and treatment of elevated homocysteine levels in high-risk populations[24-26]. The results of ongoing randomized controlled intervention trials must be available before screening for and treatment of hyperhomocysteinaemia can be recommended for the apparently healthy general population[27].

Each year about 4 million Europeans die from cardiovascular disease and its complications (CAD, peripheral artery occlusive disease, myocardial infarction, stroke, venous thrombosis)[28]. The economic burden on society and the health care system from cardiovascular disability or complications, and the treatment is quite high and getting higher rapidly in the aging population of developed countries[28,29]. Atherosclerosis is today considered a chronic condition that progresses in bouts rather than as a continuous process. Atherosclerosis is often detectable at a youthful age and

Table 4. – *Pearson's correlation coefficients (r-values) and their significance (P-values) between total plasma homocysteine values and other variables in healthy men and women.*

Variable	Men r-/P-value	Women r-/P-value	All r-/P-value
BMI (body mass index, kg/m^2)	0.011/0.927	0.116/0.453	0.036/0.702
Mean systolic blood pressure (mm Hg)	0.133/0.267	0.303/0.045	0.199/0.033
Mean diastolic blood pressure (mm Hg)	0.099/0.411	0.197/0.199	0.203/0.029
Mean fasting blood glucose	–0.188/0.120	0.088/0.585	–0.105/0.274
Mean post load blood glucose	–0.092/0.505	0.194/0.288	–0.019/0.859
Mean serum cholesterol (mg/dl)	0.061/0.614	0.208/0.197	0.080/0.406
Mean serum triglycerides (mg/dl)	0.041/0.737	0.163/0.316	0.140/0.143
Mean HDL cholesterol (mg/dl)	0.014/0.911	0.185/0.259	–0.119/0.219
Mean LDL cholesterol (mg/dl)	0.046/0.704	0.089/0.579	0.069/0.470
Mean serum creatinine (mg/dl)	0.03/0.783	0.147/0.67	0.119/0.214
C-reactive protein	0.013/0.916	0.351/0.029	0.094/0.330
Mean uric acid	0.032/0.794	0.122/0.453	0.274/0.004

* HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

therefore amenable to early, efficient prophylaxis[30]. There is therefore an increasing call for starting risk factor identification at age 20, and absolute individual risk should be known when a person turns 40[31,32]. This is very important for our country, because of the youthful structure of the population and low levels of plasma cholesterol[21]. If hyperhomocysteinaemia is found to be prevalent in our population it would be a substantial fraction of the incidence of ischaemic heart disease in our population. Therefore, we determined the reference values of Hcy levels from a sample of healthy Turkish men and women, and compared them with other reports examining other populations.

Up to now there is no consensus about reference values for plasma Hcy concentrations. In a multicentre case-control study in Europe, elevation of total Hcy (defined as > 80th percentile of controls; 12.0 μmol/l for fasting Hcy) appeared to be at least as strong a risk factor for vascular disease in women as in men, even before the menopause[11]. In 1997 Graham et al.[33] issued the report "Hyperhomocysteinemia and Vascular Disease" involving nineteen clinical centres from 11 European countries. For the purpose of certain epidemiological objectives this study chose low cut-off levels of Hcy to define mild hyperhomocysteinaemia. Although 5-15 mmol/l is given for a normal Hcy reference range, there is evidence that the risk of vascular disease may be significantly increased at levels that fall within the ostensibly normal range. The risk for vascular damage begins to rise measurably at Hcy levels as low as 10 mmol/l. Thus, there appears to be a linear progression of risk starting within what we now consider a normal level. Basing the cut-off value on the uppermost quintile of the healthy group distribution, we obtain a value of 12.26 mmol/l, comparable to the value reported by our last report[13] relating Hcy and coronary artery disease and to values obtained by Graham et al.[33] and elsewhere[10,18,19,34].

A comparative study on healthy subjects from different countries has indicated that the total serum Hcy levels vary between populations (table 5). Moghadasian et al. summarized the average Hcy levels in 35 studies involving 4338 patients with vascular diseases and 22,593 controls. The data demonstrate that control and vascular patients' plasma Hcy levels are in a wide range[18]. In a Finnish population-based study, there were no significant differences between men and women[19] and there was no significant association between Hcy and atherosclerotic disease, myocardial infarction or stroke. In these studies the mean Hcy values measured in controls were 23% lower in Japan[35] but higher in South Africa[36] than the values the authors found in Finnish controls. Recent studies show that plasma Hcy concentrations are higher in UK Indian Asians than European whites[37]. For clinicians and laboratory scientists therefore, it becomes necessary to develop reference intervals for Hcy levels, sorted according to age, sex, and ethnicity, which can be used to make decisions regarding what is a normal or abnormal Hcy level and which might be used to evaluate quantitatively the risk for diseases and disorders associated with this analysis.

In this study of healthy Turkish men and women aged between 20 and < 60 years, we observed that women had lower Hcy levels than men and levels of Hcy showed a positive association with age, for both sexes, similar to that reported in European populations. Apart from renal function, which was considered normal in this population, creatinine levels reflect muscle mass, and consequently it was not surprising that men had significantly higher creatinine levels than women. The higher Hcy levels in men may be related to their larger muscle mass, since about 75% of Hcy is formed in conjunction with creatine-creatinine synthesis. Age-related increase of Hcy levels may be dependent on reduced renal function, decreased

Table 5. – *Fasting plasma total homocysteine levels in healthy subjects from different populations.*

Source	Homocysteine level men	Homocysteine level women
Finnish population[19]	9.82 μmol/l	9.24 μmol/l
Indigenous Australian[49]	14.4 μmol/l	11.9 μmol/l
Non-Hispanic white[50]	9.6 μmol/l	7.9 μmol/l
Non-Hispanic black[50]	9.8 μmol/l	8.2 μmol/l
Mexican American[50]	9.4 μmol/l	7.4 μmol/l
Japanese population[51]	12.6 μmol/l	9.8 μmol/l
Czech men[52]	10.3 μmol/	
German men[52]	8.9 μmol/l	

activity of enzymes involved in Hcy metabolism, and decreased vitamin levels with age. There was a suggestion that the age-related increase in Hcy was stronger in women above age 60. In the post-menopausal age category, female Hcy levels, surpassed levels of men under 60 years of age. It has been suggested that this may, in part, account for the markedly increased risk of vascular disease in women after menopause. The menopause-related increase in Hcy is most likely due to hormonal changes, as no corresponding age-related changes were observed in men. A homocysteine-lowering effect by oestrogens, as suggested by some studies but not others, is thought to be responsible for lower Hcy concentrations in women compared to men, and in pre-menopausal women compared to post-menopausal women. There were significant correlations between total Hcy and blood pressure and uric acid (table 4) ($p < 0.05$) in our study group. The sex differences in uric acid and homocysteine levels may be of importance.

Factors which have been associated with increased plasma Hcy levels include older age, male sex, renal impairment, and deficiencies of folate, vitamin B12 and, less frequently, vitamin B6 (pyridoxine)[18,38]. Genetic factors may include a common mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, which codes for an enzyme which metabolises Hcy via the remethylation pathway[39,40,41]. Several possible mechanisms have been investigated to clarify the role of Hcy in provoking or aggravating vascular disease. These include endothelial injury, reduction of vascular nitric oxide production and bioavailability, a mitotic effect on smooth muscle cells, an influence on leukocyte behaviour and haemostasis, as well as oxidative modification of low-density lipoprotein[42-46]. Many of these proposed mechanisms appear to be related to oxidative stress generated by the oxidation of thiols to disulfides in the presence of reducible metal ions. These reactions produce reactive oxygen species like superoxide, hydrogen peroxide and hydroxyl radicals[47-48].

Preliminary studies indicated that elevated Hcy levels can be reduced by oral administration of B vitamins which are directly involved in Hcy metabolism. Reduction of Hcy levels in plasma is observed only when all three B vitamins (vitamin B_{12}, vitamin B_6 and folate) are supplemented. Supplementation of food with folic acid has been recommended for treatment or prevention of Hcy-related disorders, but it remains to be established whether lowering Hcy levels with vitamin therapy will decrease the risk of arterial occlusive disease.

Study limitation

The healthy Turkish population sample was obtained in the period of 2002-2003. The male population was higher than the female population. Further studies are needed with a larger sample size and of longer duration.

Conclusion

Few studies about Hcy levels of healthy persons and patients with vascular disease exist in the Turkish population. Evidence suggests that hyperhomocysteinaemia may be an important factor for vascular disease in the Turkish population which have low folate and cholesterol levels. We consider a value of 12.26 mmol/l as the upper limit for the Turkish population.

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