Association of Mean Arterial Blood Pressure with Plasma Total Homocysteine level, but Not with the Common C677T MTHFR Gene Mutation in Postmenopausal Iranian Women

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High plasma total homocystein (tHcy) level, as a source of free radicals, hydrogen peroxide may affect vascular resistance via changes in endothelial cell function. This study was designed to investigate the possible indirect effect of Hcy and common C677T Methylentetrahydrofolate Reductas (MTHFR) gene mutation (a mutant genotype revealed to be accompanied with high plasma Hcy level) on the mean arterial blood pressure (MABP).

Materials and Methods: From among 280 post-menopausal women selected on the basis of simple randomized sampling, 266 women participated in this study. Ninety-Five were known cases of hypertension under anti-hypertensive therapy with controlled blood pressure and 171 were normotensive subjects. Anthropometrics, blood pressure, plasma tHcy, plasma folic acid and vitamins B12 as well as C677T mutation in MTHFR gene were assessed for each postmenopausal female.

Results: MABP was positively and significantly associated with plasma tHcy level(r=0.230, P=0.003) but not with the common C677T MTHFR gene mutation. In regression analysis, plasma tHcy was the only predictor of MABP (R^2=0.05, P=0.004). High plasma tHcy level increased hypertension risk in comparison to normal plasma tHcy level (OR=3.11, CI=1.07-9.18) whereas the mutant MTHFR genotype, TT allele, in comparison to the wild MTHFR genotype, CC allele, did not.

Conclusions: Mean arterial blood pressure was significantly associated with plasma tHcy level but not with the common C677T MTHFR gene mutation.

Introduction

Increased arterial blood pressure is the consequence of alterations in relationship between blood volume and peripheral vascular resistance. In regulation of blood pressure, multiple systems including central and peripheral adrenergic, hormonal and vascular systems are involved.

This regulation is the result of complex interaction among these systems, of which vasculature has a critical role as the others do.

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Endothelial cells form the endothelium that lines the entire vascular system and plays a fundamental role in the control of blood pressure by modulation of blood flow and vascular resistance achieved by the release of vasoactive factors including vasoconstrictor (endothelin) and vasodilators (nitric oxide, prostaglandin). Alteration in the functional state of endothelial cells termed endothelial dysfunction leads to impaired endothelium dependent vasodilatation, reduced synthesis of nitric oxide, elevated level of endothelin, and production of free radicals[1-2] which can finally lead to alteration in blood pressure. Among well documented risk factors for endothelial cell damage such as hypertension, hyperlipidemia, cigarette smoking, high plasma homocysteine (Hcy) level is more recently being investigated. Cross sectional and retrospective case-control studies and one large prospective observational study showed the strongest association between hyperhomocysteinemia and vascular risk.[3-7] Homocysteine is a key junctional metabolite in methionine metabolism; either a genetic defect in one of the enzymes of its metabolism, Methylentetrahydrofolate reductase (MTHFR) and Cystathionine β-synthase (CβS), or nutritional deficiency of one or more vitamins (folic acid, vitamin B12 or B-6) that participate in its metabolism can lead to metabolic disruption and potentially hyperhomocysteinemia.[8] Hcy in high plasma levels is a source of hydrogen peroxide and homocysteine thiolactone (a product of autooxidation of Hcy), harmful free radials which damage endothelium;[9-15] therefore, it is clear to postulate that high plasma Hcy level causing endothelial cell damage leads to alteration in blood pressure.

The MTHFR enzyme catalyzes reduction of methylentetrahydrofolate to methyltetrahydrofolate to perform methyl group for re-methylation of Hcy to methionine in the re-methylation pathway of Hcy metabolism. Common C677T MTHFR genotype mutation, in which a C→T substitution at base pair 677occurs and leads to exchange of an alanine to a valine,[16] is associated with reduced activity of the enzyme and, therefore, with high plasma Hcy level[17-19] and higher risk of cardiovascular disease. Controversial results of previous studies[20-22] as well as the racial dependency of arterial blood pressure and Hcy metabolism [23] were the reasons we undertook this study.

Materials and Methods

On the basis of smiple randomized sampling 280 subjects were selected from among 306 postmenopausal women whose addresses and telephone numbers were available from a previous study in which postmenopausal women were chosen randomly by telephone number from fourteen areas of Shiraz city. Ninety-five postmenopausal women who participated were known cases of hypertension and one hundred seventy one were normotensive subjects (blood pressure<135/85).

Three cases were unrecognized hypertensive subjects and six were diabetic, and on the basis of exclusion criteria were excluded. We considered diabetes as an exclusion criterion to find a clearer association of plasma tHcy and common C677T MTHFR gene mutation with MABP. The reasons for exclusion of these cases were studies[24-28] in which possible association of diabetes with Hcy or common C677T MTHFR mutation was found. Shiraz city is divided to fourteen areas on the basis of the first three digits of telephone numbers. Data on past medical history and drug therapy and on variables such as water pipe smoking, physical activity, alcohol, vitamin supplementations and dietary intake were obtained from all study participants at baseline. The local ethics committee approved this study and written informed consent was obtained from all subjects

Exclusion criteria:

Hypertension caused by a definable etiology (secondary hypertension), diabetes mellitus, and new cases of hypertension under no anti-hypertensive therapy, and postmeno-

pausal women with recognized underlying disorders were exclusion criteria.

Inclusion criteria:

Postmenopausal women with no recognized underlying disorder or with hypertension controlled by anti-hypertensive therapy (blood pressure<135/85) were included.

Blood sampling and polymerase chain reaction (PCR):

Postmenopausal women presented in a fasting state, between 7 to 8 o'clock in the morning, and blood pressure was measured ten minutes after arrival, with the arm in a resting position on table, free of clothing and at the level of the heart. Blood samples were taken and centrifuged within thirty minutes; then plasmas were frozen at -20°C. Glomerular filtration rate (GFR) was calculated by the Cockcroft-Gault formula $[(140\text{-age})\times$ lean body weight (kg)]$\times 0.85/$[plasma creatinine (mg/dL)$\times 72$] and mean arterial blood pressure was calculated as follows: [systolic blood pressure (+Hcy) (diastolic blood pressure$\times 2$)]/3.

Plasma total homocysteine as well as plasma folic acid and Vitamin B12 were analyzed by commercially available enzyme immunoassay kits (DRG, Diagnostic, USA) and simultaneously using commercially available radio-assay kits (Simultrac -SNB-CNI, Pharmaceutical, USA), respectively. Creatinine was measured using commercial kits and the Cobass autoanalyser.

Genomic DNA was prepared from peripheral blood. The methods used for polymerase chain reaction restriction fragment length polymorphism of the genomic DNA.

Data analysis:

The results of the variables are shown as mean±SD. All tests were two-tailed and a P-value less than 0.05 was considered significant. Correlation of MABP with other variables was assessed by Pearson and Spearman Bivariate coefficient correlation analysis,. The independent sample student t-test was used to compare means of plasma tHcy and GFR between hypertensive and normotensive groups.

Regression analysis and odd ratio were performed to assess MABP predictors and hypertension risk in conditions with hyperhomocysteinemia and MTHFR TT genotype in comparison to normohomocysteinemic state and MTHFR CC genotype, respectively. Because there are no general cut off points to categorize people with the normal, marginal and high plasma tHcy level, three categories of plasma tHcy were created on the basis of tertiles of plasma tHcy in normotensive subjects; normal or lowest terrile (<11), marginal or middle tertile ($11 \le \text{Hcy} < 17$) and high plasma tHcy or highest tertile (≥ 17). On the basis of tertiles of plasma folate in normotensive subjects, three categories of plasma folate were also created: normal or lowest terrile (< 3.5), marginal or middle tertile ($3.5 \le \text{folate} < 6.85$) and high plasma folate or highest tertile (≥ 6.85).

Results

Anthropometrics and biochemical parameters of postmenopausal women are summarized in Table 1. Frequencies of mutant alleles, CT and TT were 36.1% and 9.5%, respectively and frequency of the CC allele was 55.4%.

Among all the variables of age, years since menopause, age at menopause, height, weight, body mass index (BMI), GFR, creatinine, plasma tHcy, folate and vitamin B12, the only variable with statistically significant association with MABP was plasma total homocysteine (Pearson's correlation r=0.230, P=0.003). Water pipe smoking and MTHFR gene mutation (C677T) showed no statistical significant correlation with MABP in Bivariate spearman's correlation (r=0.060 p=0.449; r=-0.012, p=0.877).

Systolic and diastolic blood pressure in association with mean arterial blood pressure showed a significant correlation(r=0.765, p=000 and r=0.840, p=000, respectively), but in association with plasma tHcy, only systolic blood pressure showed statistical correlation (r=0.222, p=0.004). Plasma tHcy was nega-

tively and significantly associated with plasma folic acid and vitamin B12(r=-0.362, p=0.000 and r =-0.332, P=0.000, respectively), Table 2 shows correlates of MABP, systolic and diastolic blood pressures.

Linear regression analysis showed plasma tHcy as the only predictor of MABP among all variables evaluated (age, years since menopause, age at menopause, height, weight, BMI, GFR, Plasma folate, vitamin B12, plasma tHcy, creatinine, water pipe smoking and C677T MTHFR gene mutation) (R^2=0.05, P=0.004) (Table 3).

Mean plasma tHcy levels differed significantly between hypertensive and normotensive groups (15.71±5.21µmol/L vs. 13.38 ±3.88 µmol/L P=0.008, respectively),

whereas GFR did not. (64.84±18.24 vs. 68.98±17.76, p= 0. 169, respectively).

The frequency of hypertension increased from 25% to 44.6% from the lowest tertile of Hcy to the highest; the frequency of normal blood pressure however decreased from 75% to 55.4%.

Mean plasma tHcy, folate and vitamin B_{12} level among three alleles of 677MTHFR genotype, CC, CT, and TT, (Anova analysis)

Mean plasma tHcy, folic acid and vitamin B_{12} did not differ statistically different between the three alleles. Of 677MTHFR genotype. Table 4 shows plasma Homocysteine in relation to folate and C677TMTHFR genotypes.

Table 1. Characteristics of study subject; anthropometrics and biochemical parameters

Characteristics	Mean	SD
Age	61.98	7.54
Age at menopause	49.67	5.08
Years since menopause	11.30	7.64
BMI, kg/m^2	29.09	15.87
Creatinine µmol/L	81.08	12.86
GFR, mL/min	60.98	16.23
Albumin (g/L)	4.71	0.41
Geometric mean plasma total homocysteine, µmol/L	14.26	4.46
Geometric mean Plasma folate, nmol/L	5.84	4.89
Vitamin B12, pmol/L	254.23	158.89
Mean arterial blood pressure, mmHg	93.34	2.35

BMI and GFR indicate body mass index and glomerular filtration rate.

Table 2. Correlates of mean arterial, systolic and diastolic blood pressures

Variables	Hypertension risk r	P	SBP[†] r	P	DBP[‡] r	P
Age	0.307	0.000	0.214	0.005	0.014	0.860
Age at menopause	0.147	0.057	0.089	0.249	0.014	0.856
Years since menopause	0.225	0.003	0.076	0.329	0.033	0.669
Log of BMI	0.101	0.199	0.90	0.256	0.176	0.025
Log of Hcy	0.201	0.009	0.137	0.07	0.085	0.292
Log of Plasma folate	-0.072	0.254	-0.106	0.176	-0.112	0.151
Plasma Vit.B12	-0.132	0.091	-0.140	0.072	-0.112	0.181
Systolic blood Pressure	0.332	0.<001	0.457	0.000		
Diastolic blood pressure	0.205	0.<001	0.457	0.<001		
Water pipe smokeing	0.058	0.457	0.046	0.553	0.108	0.164
Creatinine	0.068	0.352	0.017	0.822	-0.078	0.313
GFR	-0.136	0.079	-0.113	0.147	0.159	0.041
MTHFR Genotypes	-0.030	0.699	-0.031	0.694	-0.038	0.624
Albumin	0.151	0.051	-0.128	0.099	-0.058	0.458

†=Systolic blood pressure, ‡=Diastolic blood pressure.

Table 3. Predictor of mean arterial blood pressure

Independent variable	Coefficient β	Adjusted R^2	P
Constant	85.134		
Total homocysteine	0.579	0.05	0.004

The coefficient β (95% confidence interval) represents changes per variable unit.
The adjusted–R^2 represents the explained variance of hypertension per added variable.

Table 4. Plasma homocysteine in relation to folate and C677T MTHFR genotypes

	TT	CT	CC	P Value
Serum folate lowest tertile	18.30±5.32	16.82±1.62	16.55±5.59	0.537
Serum folate middle tertile	15.7±0.53	13.73±3.53	13.07±3.31	0.196
Serum folate highest tertile	14.19±0.15	13.1±3.25	12.29±3.56	0.440

Table 5. Crude and adjusted odd ratio for hypertension in normal, marginal and high plasma homocysteine levels

	Normal	Marginal	High
Crude	1	1.08 (0.42-2.77)	3.11[*] (1.07-9.13)
+Age +BMI +Years since menopause	1	0.97 (0.61-1.55)	2.26[*] (1.43-4.05)

Values are crude and adjusted odd ratio (95% confidence interval). The crude odds ratios were adjusted for other related variables to hypertension age, height and years since menopause. *P<0.05.

Hyperhomocysteinemia increased hypertension risk in comparison to normohomcysteinemic state (OR=3.11, CI=1.07-9.18), but the 677MTHFR TT genotype did not (OR: 0.72, CI=0.17-2.72) in comparison to 677MTHFR CC genotype. The hypertension risk in hyperhomocysteinemic state remained significant, even after adjustment for other related variables to hypertension including age, years since menopause and height (OR=2.26, CI=1.43-4.05), (Table 5).

Discussion

Our study demonstrates that mean arterial blood pressure is significantly and positively associated with the plasma tHcy level but not with the common MTHFR gene mutation (C677T). It also indicates that while hyperhomocysteinemia increases hypertension risk the 677MTHFR TT genotype does not.

Our study finding of the hypertension relationship with plasma tHcy is similar to that of the Chamber et al study[29] in which a graded relation between tHcy and hypertension has been found independent of BMI, waist /hip ratio, fasting glucose, TG and HDL cholesterol. A study in Srilanka of 86 hypertensive patients compared with 82 normotensive controls also determined increased tHcy in the hypertensive group.[30] These studies as well as our investigation determine that association of Hcy with hypertension may be independent of racial composition.

Among our study results, the C677T MTHFR gene mutation is neither associated with MABP nor increases hypertension risk. This is inconsistent with the plasma tHcy relationship with MABP and hypertension risk if we consider C677T MTHFR gene as a predictor of plasma Hcy level.

About 50 studies have now been published on the association between cardiovascular disease and C677T MTHFR gene mutation; just over half of these show a significant association, whereas the remainder often show a trend towards an association. Among vari-

ous explanations proposed for this inconsistent association of C677T MTHFR polymorphism and cardiovascular disease, folate status has been suggested as a possible explanation which was not taken into account in some previous studies when association was calculated.[31] In the Vander Put et al[32] and Harman et al studies,[33] folate status as an important determinant of Hcy level, has been shown to prevent the Hcy elevating effect of the C677T MTHFR TT genotype when its status is adequate.

In our study subjects, plasma tHcy level showed no statistically significant difference among three different polymorphisms of C677T MTHFR, which can indicate the inconsistent association of C677T MTHFR polymorphism with MABP and hypertension risk and explain the preventive influence of adequate folate status on Hcy elevating effect of the C677T MTHFR gene mutation. This result may be due to high consumption vegetable rich in folate in our population. This is also confirmed by our finding of no statistically significant difference in folate level in three different polymorphisms of C677T MTHFR. This preventive impact of adequate folate status on the Hcy elevating effect of C677T MTHFR mutation could therefore be a possible reason for the relatively low prevalence of cardiovascular disease in Italy where the TT genotype is very common.[34]

There are studies which show the effect of renal function on Hcy level[35-37] and the Brattstrom et al study suggested that the modest Hcy increase found in patients with cardiovascular disease was an epiphenomenon, a consequence of impaired renal function, and not a direct cause. In our study, for evaluation of this possibility, the mean of GFR in both groups was measured and no statistically significant difference between hypertensive and normotensive groups was found. This result shows that different plasma tHcy levels found between groups are not due to the possible effect of renal function on Hcy level.

Smoking is not very common among Iranian women, but in postmenopausal women, water pipe, a type of tobacco smoke, is more popular than cigarette smoking. In our study all 64 women smokers were water pipe smokers (5-10 minutes use each time, weekly use of 1-3 times). Water pipe smoke, considerning the amount used, neither increased hypertension risk nor was it associated with MABP. Since no studies has examined this association, further studies investigating this possibility should be conducted in other populations.

Tobacco smoking has been shown to have multiplicative interactions with other cardiac risks, such that the increment in risk produced by smoking in individuals with hypertension and hyperlipidemia was greater than the increment in risk produced by smoking for individuals without these risk factors. The hypertension risk in hyperhomcysteinemic state with and without water pipe smoking was examined to investigate the abovementioned interaction of water pipe as a tobacco smoke with hyperhomocysteinemia as a cardiac risk factor. Increased risk of hypertension in the hyperhomocysteinemic state with water pipe smoking (OR: 2.43, CI: 0.47-13.10) in comparison to the hyperhomocysteinmic state without water pipe smoking (OR: 2.26, CI: 0.61-8.51) confirmed the tobacco smoke interaction with hyperhomocysteinemia. We have no data on the erythrocyte folate status of this group of postmenopausal women as it has been suggested as being a longer and better marker for the body's folate status[38] than plasma folate. However, correlates of mean arterial, systolic and diastolic blood pressures showed no significant relation between plasma folate mean arterial, systolic and diastolic blood pressures in this study.

Overall the study indicates that plasma tHcy is an important predictor of mean arterial blood pressure and hypertension risk; it also demonstrates that adequate folate status has a preventive impact on the elevating effect of the C677T MTHFR mutation.

Acknowledgment

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