Association of Plasma Homocysteine, Serum Folic Acid and Vitamin B12 Concentrations and MTHFR C677T Polymorphism with Preeclampsia

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OBJECTIVE: This study aims to evaluate the association between preeclampsia and plasma homocysteine, serum folic acid, vitamin B12 concentrations and MTHFR C677T polymorphism.
STUDY DESIGN: This study was a case-control study including 20 pregnant females with preeclampsia and 30 healthy normotensive pregnant (ages 18-40) females. Plasma homocysteine, serum folic acid and vitamin B12 concentrations were measured in all patients in the third trimester of pregnancy and MTHFR C677T polymorphism was also analyzed.
RESULTS: The risk of preeclampsia in patients with homocysteine concentrations >8.65 µmol/L increased 8-fold as compared to homocysteine concentrations <6.19 µmol/L. While the mean plasma homocysteine concentration (8.65±2.05 µmol/L vs. 6.19±1.52 µmol/L, p<0.001), was high in the preeclampsia group as compared to controls, the mean serum folic acid concentration was significantly low (11.49±8.96 ng/ml vs. 15.15±6.7 ng/ml, p= 0.020). No significant difference was noted between the groups regarding mean serum vitamin B12 concentrations (241.1±111.7 pg/ml vs. 236±111.1 pg/ml, p= 0.879) and MTHFR C677T polymorphism including MTHFR gene TT/CT/CC genotypes.
CONCLUSION: Elevated third trimester plasma homocysteine concentrations were associated with increased risk of preeclampsia. This association was more pronounced in our study which may also be related to synergistic effect of the coexistent folic acid deficiency. MTHFR C677T polymorphism could not alone explain the hyperhomocysteinemia in patients with preeclampsia.
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Key Words: Folic acid, Homocysteine, MTHFR C677T polymorphism, Preeclampsia, Vitamin B12

Preeclampsia is a maternal syndrome that occurs only during pregnancy and remains among the leading causes of maternal and perinatal morbidity and mortality worldwide.[1] The etiology of preeclampsia is unknown. Potential etiologies include abnormal trophoblast invasion of uterine blood vessels, immunological intolerance between fetoplacental and maternal tissues, maladaptation to the cardiovascular or inflammatory changes of pregnancy, dietary deficiencies, and genetic abnormalities.[2] In the pathogenesis of preeclampsia, endothelial cell dysfunction and inflammation have been proposed as the central feature.[3]

Preeclampsia is a disorder with a strong heritable component, and a family history of preeclampsia is associated with a fourfold increased risk of severe preeclampsia.[4] In addition, maternal metabolic disturbances might contribute to the aberrant endothelial function and subsequent clinical manifestations of preeclampsia and eclampsia, since diseases known to be related with endothelial damage (e.g., diabetes and chronic hypertension) predispose women to the development of preeclampsia.[5,6]

Elevated plasma homocysteine is a risk factor for endothelial dysfunction and vascular diseases such as atherosclerosis and occlusive vascular disorders.[7] Hyperhomocysteinemia also has been associated with complications in pregnancy such as neural tube defects, repeated miscarriages, placental abruption, fetal death and intrauterine growth retardation.[8] The concentration of plasma homocysteine is regulated by several factors including genetically determined metabolic enzyme alterations, nutritional status, certain drugs, age, and pregnancy. Plasma homocysteine concentrations decrease during pregnancy[9] and are closely dependent on vitamin B12 and folic acid intake.[8] One of the genetic bases of hyperhomocysteinemia is suggested to be caused by a polymorphism in the 5, 10-methylenetetrahydrofolate reductase (MTHFR) gene where homozygosity for the 677(C→T) substitution results in a reduced MTHFR enzyme activity, and subsequently elevated homocysteine concentrations.[10]

In this study we aimed to clarify the association between plasma homocysteine, serum folic acid and vitamin B12 concentrations and preeclampsia and evaluate whether MTHFR C677T polymorphism may be a contributing factor in preeclampsia

Material and Method

This case-controlled study was carried in the obstetrics and gynecology department of the Ankara University Hospi-

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tal between May 2002 and January 2004. Twenty preeclamptic and 30 healthy pregnant subjects were included in the study. Preeclampsia was diagnosed in the presence of a blood pressure ≥140/90 mmHg on at least two occasions more than 6 hour apart, and a proteinuria higher than 300 mg/24 hour, after the 20th week of pregnancy. The study was performed with the approval of the Institutional Ethical Committee of the School of Medicine. Written consent for participation was obtained after the design and aim of the study was explained to all participants.

All of the women were primigravida of whose age ranged between 18 and 40, and none received any drug except for iron supplementation. Exclusion criteria were as follows: special diets implying folic acid or vitamin B12 consumption higher or lower than normal intakes in our geographical area (assessed by a nutritional questionnaire), altered renal function, diabetes or chronic diseases such as chronic hypertension, treatment with antifolate or antiepileptic drugs and twin pregnancies. The patients were free from any infectious or chronic diseases and had no history of alcohol use, cigarette smoking or vitamin deficiency. No prior history of arterial or venous thrombosis was reported. The women who received multivitamin supplements including folic acid, vitamin B12 and zinc prior to the study and also women who had blood transfusions were excluded from the study. We have information about the resolution of the clinical symptoms of preeclampsia after delivery in order to rule out women with chronic hypertension and the superimposition of preeclampsia.

A nutritional questionnaire was used to record a dietary history (3 day recall). The nutritional status of the women were analyzed by the Nutrition Department of Ankara University School of Medicine and were divided into two categories as of "poorly nourished" and "well nourished" groups according to the intake of calories, percentage of total and unsaturated fats, total protein, simple and complex carbohydrates in the diet with a computerized nutrition program BeBiS 1.0.[11] Biochemical analyses were performed in all patients during the 3rd trimester of pregnancy. (Table I). BMI measurements in the study were made by from self reports of pre-pregnancy weight data.

After an overnight fast, venous blood samples were drawn for determination of plasma homocysteine, serum folic acid and vitamin B12 concentrations and for analysis of MTHFR C677T polymorphism. Plasma from EDTA anticoagulated tubes were used to measure the total L-homocysteine plasma concentration with fluorescence polarization immunoassay in the IMx® analyzer (IMx, Abbott Laboratories, Abbott Park, Illinois, USA).[12] The serum folic acid and vitamin B12 concentrations were measured by radioimmunoassay by means of a Simul TRAC-SNB kit from ICN Pharmaceuticals (PhRMA), CA, USA. For MTHFR analysis, genomic DNA was isolated from 5 ml peripheral blood samples by standard phenol: chloroform extraction.[13] Polymerase chain reaction was performed in a 20-ml total volume containing 0.1 mg genomic DNA, 10 pmol of each primer, 0.2 mM of each dNTP, 10 mM Tris, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.5 U Taq polymerase. PCR conditions were as follows: initial denaturation at 94^0C for 5 minutes and then 35 cycles of 94^0C for 30 seconds, 63^0C for 30 seconds and 72^0C for 1 minute followed by a final extension at 72^0C for 5 minutes. Restriction digestion was performed in a total volume of 15-µl reaction mixture containing 10 µl PCR product and 1 U HinfI restriction endonuclease. Reaction mixtures were incubated at 37°C overnight and the genotypes were determined by agarose gel electrophoresis of restriction digests in 2% agarose gel containing 0.5 µg/ml ethidium bromide.[14]

Table 1. Clinical characteristics and nourishment status of the study group and control subjects.

	Preeclampsia (n= 20)	Controls (n=30)	P value
Maternal age	27.4±4.6	25.2±3.9	0.079
BMI (kg/m^2)	22.2±2.5	22.1±2.0	0.858
Gestational age (week) *	232.1±17.5	241.8±9.9	0.084
Birth weight (gram)	2582±684	3260±316	<0.001
APGAR 1 min.	6.4±1.9	8.0±0.7	<0.001
APGAR 5 min	8.5±2.1	9.7±0.5	<0.001
Well nourished	11 (%55)	18 (%60)	> 0.05
Poorly nourished	9 (%45)	12 (%40)	> 0.05

BMI: Body mass index.

*Pregnancy day the samples were withdrawn (Dates).

All values given as mean ± SD

Statistical Analysis

Continuous variables were evaluated by using Student's t test or Mann-Whitney U test, where applicable. Differences among three genotypes for homocysteine, folic acid, vitamin B12 were evaluated by Kruskal-Wallis variance analysis, and when p-value was significant, multiple comparison tests were used.[15] Categorical variables were tested by Chi-square test or Fisher's exact test, where applicable. $P< 0.05$ was considered as statistically significant.

Results

Both groups were similar with respect to age, body mass index and gestational age (Table 1). Birth weight, gestastitional age at birth, 1 and 5-minute Apgar scores were significantly lower in the preeclampsia group with respect to the controls.

Nutrition history was similar between the preeclampsia and control group. ($\chi 2= 0.12$, $p= 0.73$). Eleven of the 20 pa-

tients (55%) in the study group, and 18 of the 30 control subjects were well nourished (Table 1). Mean plasma homocysteine concentration was significantly higher in the preeclampsia group than in the control group (8.65±2.05 µmol/L vs. 6.19±1.52 µmol/L respectively, p<0.001) (Table 2). Plasma homocysteine concentrations >8.65 µmol/L increased the risk of preeclampsia 8-folds as opposed to homocysteine concentrations < 6.19 µmol/L. Mean serum folic acid concentration in the preeclampsia group was significantly lower than in the control group (15.15±6.7 ng/ml and 11.49±8.96 ng/ml respectively, p=0.02). Serum concentrations of vitamin B12 were similar in the study and the control group (241.1±111.7 pg/ml and 236±111.1 pg/ml respectively, p=0.879).

Table 2. Plasma homocysteine, serum folic acid and vitamin B12 concentrations in the study group and control subjects.

	Preeclampsia (n= 20)	Controls (n=30)	P value
Homocysteine (µmol/L)	8.65±2.05	6.19±1.52	<0.001
Folic acid (ng/ml)	11.49±8.96	15.15±6.7	0.020
Vitamin B12 (pg/ml)	236± 111.1	241.1±111.7	0.879

All values given as mean ± SD

Table 3. Distribution of the methylenetetrahydrofolate reductase genotypes in preeclamptic patients and control subjects.

	MTHFR C677T polymorphism		
	CC genotype	CT genotype	TT genotype
Preeclampsia (n= 20)	12 (%60.0)	6 (%30.0)	2 (%10.0)
Controls (n=30)	21 (%70.0)	7 (%23.3)	2 (%6.7)

No significant difference was noted in the distribution of the methylenetetrahydrofolate reductase genotypes in preeclamptic and control subjects ($\chi 2$= 0.55, p= 0.76) (Table 3). Frequency of MTHFR CC genotype in the preeclampsia and control group was 12/20 (60%) and 21/30 (70%) resepectively. MTHFR CT was detected in 6/20 (30%) of the preeclamptic patients and 7/30 (23.3%) of the control subjects MTHFR TT genotype was found in 2/20 (10%) of the study patients and 2/30 (%6.7) of the control subjects. Carrying a MTHFR TT genotype increased the risk of preeclampsia 1.75 times when compared with those with MTHFR CC genotype (95% CI 0.22-14.1), though not statistically significant, (p=0.63). Carrying a MTHFR CT genotype increased the risk of preeclampsia 1.5–fold when compared to those having a CC genotype (95% CI; 0.41-5.5, p=0.54).

No significant differences were found among MTHFR CC/CT/TT genotypes regarding homocysteine, folic acid and vitamin B12 concentrations (Table 4). Though not statistically significant, in subjects who had MTHFR TT genotype, plasma homocysteine concentrations were lower and serum folic acid concentrations were higher when compared with those having other genotypes.

Discussion

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine. In the general population, hyperhomocysteinemia has an estimated prevalence of 1 in 70.[16] Elevated concentrations of homocysteine are associated with an increased risk of atherosclerosis and thrombosis.[17] During oxidation of the sulfhydryl group of homocysteine, superoxide anion radical (O_2-) and hydrogen peroxide (H_2O_2) are generated, and these oxygen-derived molecules are believed to account for facilitation of thrombin generation and endothelial cytotoxicity.[13,16] Elevated homocysteine also promotes endothelial cell dysfunction and subsequent atherogenesis via its role in increasing nitric oxide production.[18]

In the present study, high maternal plasma homocysteine concentrations were associated with an increased risk of preeclampsia. Preeclampsia was found to be increased in women with average homocysteine concentrations of 8.65 µmol/L compared to women with average homocysteine concentration of 6.19 µmol/L. In a similar case control study by Sanchez et al. among 125 preeclamptic and 179 control patients, women with homocysteine concentrations above 9.1 µmol/L experienced a fourfold increase in the risk of preeclampsia as compared with women with values below 5.3 µmol/L in the third trimester.[19] Similarly Lopez-Quesada et al. demonstrated that pregnant women with hyperhomocysteinemia (>10.5 mmol/L) had a 7.7-folds higher risk for preeclampsia compared with normal controls in the third trimester.[20] In addition, several studies have reported that maternal plasma homocysteine is significantly elevated in women with overt preeclampsia when compared to normal pregnant women.[21,22]

In our study, high third trimester plasma homocysteine concentrations were associated with a higher risk of preeclampsia as demonstrated by previous studies.[19-22] However, conflicting with the results of those studies, we observed lower serum folic acid concentrations in the third trimester in preeclampsia cases.[20,22] We believe that lower than normal serum folic acid concentrations might have contributed to the higher homocysteine concentrations in the study group, leading to a synergistic action with hyperhomocysteinemia and caused augmentation of the preeclamptic effect.

Several factors may contribute to elevated plasma homocysteine concentrations in preeclampsia, including decreased folic acid intake or oxidation, genetic polymorphisms,[23] impaired placental amino acid transport,[24] and decreased delivery of homocysteine from liver to kidneys due to decreased

Table 4. Comparison of homocysteine, folic acid and vitamin B12 concentrations in different MTHFR genotypes.

	CC genotype	CT genotype	TT genotype	P value
Homocysteine(µmol/L)	6.9±2.0	7.1±1.5	7.9±2.6	0.55
Folic acid (ng/ml)	15.0±8.6	11.5±5.5	10.0±5.2	0.27
Vitamin B12 (pg/ml)	245.1±106.8	217.1±112.0	261.3±154.8	0.75

All values given as mean ± SD

hepatic blood flow. Changes in renal handling do not appear to contribute to the increase in plasma homocysteine in preeclampsia.[25] Although Murphy et al., refutes the reduction in plasma homocysteine secondary to folic acid supplementation, hemodilution, and decrease in serum albumin in normal pregnancies,[26] the significantly decreased plasma volume in preeclampsia may contribute to increased homocysteine concentrations as well.

It is unclear whether hyperhomocysteinemia follows or precedes the development of preeclampsia. In a prospective study by Sorenson et al. second trimester elevation of homocysteine was associated with a 3.2-folds increased risk of preeclampsia.[27] Cotter et al. analyzed plasma homocysteine concentrations in 56 severe preeclamptic and 112 healthy subjects, and 71 non-severe preeclamptic and 142 control patients. They concluded that an elevated plasma homocysteine concentration in early pregnancy might increase the risk of developing severe preeclampsia by almost threefold and non-severe preeclampsia by fourfold.[28,29] They noted that homocysteine concentrations may even be elevated before pregnancy, causing endothelial cell dysfunction as an ongoing process, and pregnancy serves as a predisposing factor to some other secondary influence leading to the development of preeclampsia.

Maternal serum folic acid deficiency is a probable risk factor for placenta-mediated diseases, such as preeclampsia, spontaneous abortion and placental abruption.[30] In our study, we observed that low maternal serum folic acid concentrations were associated with an increased risk of preeclampsia, whereas other studies reported similar or higher maternal serum folic acid concentrations in patients with preeclampsia compared to uncomplicated pregnancies.[20,22,31] High folate concentrations in preeclamptic patients may be due to ingestion of a rich folate diet during pregnancy and folate supplementation during the first trimester.[20] Although in our study nutrition history of preeclampsia and control cases was similar, lower serum folic acid concentrations were encountered in patients that developed preeclampsia. Alterations in the absorption and pharmacodynamics of folic acid, rather than nutritional status, might cause low serum folic acid concentrations in patients developing preeclampsia.

Maternal vitamin B12 deficiency is less well defined as an important risk factor for preeclampsia.[30] Although many studies reported decreased vitamin B12 concentrations in cases with preeclampsia when compared with uncomplicated pregnancies,[32] some showed no change.[21] No direct association was found between serum vitamin B12 concentration and the risk of severe preeclampsia in the early pregnancy (15 weeks).[29] In our study vitamin B12 serum concentrations in the study and the control group were similar, and we suggest that that no direct correlation exists between the risk of developing preeclampsia and maternal serum vitamin B12 concentrations in the third trimester.

No significant difference was detected between MTHFR CC/CT/TT genotypes and homocysteine, folic acid and vitamin B12 concentrations. However patients with MTHFR TT genotypes had lower folic acid concentrations and elevated homocysteine concentrations when compared with other genotypes. The small numbers of the study may indicate lack of power and thus possible associations of MTHFR genotypes with other parameters could not have become evident. This is a limitation of the study.

Eventhough some studies have revealed an association between preeclampsia and MTHFR gene polymorphism,[23,32,33] others failed to define such an association[31,34-39] We did not identify an increased frequency of MTHFR C677T homozygocity or heterozygocity among mothers with preeclampsia in our study groups. Having a MTHFR TT or CT genotype was associated with statistically insignificant risks for the development of preeclampsia, which suggests that although MTHFR polymorphism may contribute to increase in homocysteine concentrations, could not be identified as a direct cause of hyperhomocysteinemia, therefore it seems very unlikely that single polymorphism is having a significant effect in preeclampsia. We need large scale prospective randomized studies to make a clearer conclusion regarding the association between MTHFR polymorphism and hyperhomocysteinemia in patients with preeclampsia.

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