Elevated plasma total homocysteine in preeclampsia

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ABSTRACT

الأهداف: التحقق من العلاقة المحتملة بين ارتفاع الهيموسيستين وحالات مقدمة الارتعاج.

الطريقة: أجريت الدراسة على مجموعة التحكم في الفترة ما بين مايو 2004م إلى أغسطس 2006م، في قسم النساء والولادة بمستشفى جام – إيران. شملت الدراسة (75) مريضة تعاني من حالة مقدمة الارتعاج. سبعة وثلاثون امرأة تعاني من مقدمة الارتعاج البسيط، (37) امرأة تعاني من مقدمة الارتعاج الشديد و(40) امرأة سليمة (مجموعة التحكم) بدون مضاعفات الحمل. تم تحديد الهيموسيستين في مصل الدم الكامل عن طريق الأنزيم المرتبط للماص المناعي.

النتائج: كانت المجموعات الثلاثة متشابهة في العمر والجسم. لدى المريضات اللواتي يعانين من مقدمة الارتعاج الشديد مستويات فعلية لمصل الدم أعلى (13.8±7mg/l) من ذوات الحمل الطبيعي (8.8±2.8mg/l) والنساء اللواتي يعانين من مقدمة الارتعاج البسيط (10.4±2.3 mg/l) (p<0.05).

خاتمة: تبين أن النساء اللواتي تعرضن لمقدمة الارتعاج الشديد تكون مستويات هيموسيتسين في مصل الدم لديهن أعلى من النساء اللواتي بقي ضغط دمهن على شكل طبيعي أثناء الحمل.

Objectives: To investigate the possible association between hyperhomocysteinemia and preeclampsia.

Methods: A case-control study was carried out in the Departments of Obstetrics and Gynecology of the Ghaem Hospitals in Mashhad University of Medical Sciences, Mashhad, Iran from May 2004 to August 2006 and included 75 preeclamptic patients, 37 women with mild preeclampsia, and 38 women with severe preeclampsia, in addition we included 40 controls without pregnancy complications. Plasma total homocysteine was determined in all subjects by enzyme linked immunosorbent assay.

Results: The 3 groups were similar in age and body. Patients with severe preeclampsia had significantly higher mean plasma levels (13.8±7mg/l) than normal pregnant women (8.8±2.8mg/l) and mild pre-eclamptic women (10.4±2.3mg/l)(p<0.05).

Conclusion: Women who developed severe preeclampsia have higher plasma homocysteine levels than women who remain normotensive throughout pregnancy.

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Preeclampsia represents one of the most important complications of pregnancy (5-7% of low-risk pregnancies), but little is known of its etiology.[1] It may be defined as a pregnancy specific occlusive vascular disorder characterized by endothelial cell dysfunction and increased platelet aggregation. Hyperhomocysteinemia has been hypothesized to be associated with this placental micro vascularization disease.[2] Plasma total homocysteine (tHcy; the sum of all homocysteine forms, which generate this amino acid by reduction) depends on many physiological, pathological, and genetic determinants, which are closely interrelated to each other.[2-4] Plasma tHcy concentrations decrease during pregnancy,[5] and are closely dependent on Vitamin B (folate, Vitamins B12 and B6, riboflavin) intake.[2-4] Although changes in tHcy have been reported in northern European countries,[5] and America,[6] there are limited data in the literature on plasma tHcy concentrations during pregnancy,[7] and on the occurrence of hyperhomocysteinemia in preeclampsia in our geographical area. Preeclampsia, intrauterine growth restriction, and spontaneous miscarriage have been related to individual amino acid alterations or abnormal amino acid profiles. Increased levels of lipid peroxides, coupled with decreased activity of antioxidants in women with preeclampsia, has raised the possibility that markers of oxidative stress might

predict preeclampsia. Hyperhomocysteinemia has been associated with preeclampsia by many authors,[2,8] who have suggested that homocysteine might play a role in promoting endothelial dysfunction. Therefore, the goal of this study was to evaluate the possible association between plasma total homocysteine and severity of preeclampsia in pregnant women.

Methods. A case control study was designed to compare the plasma concentration of homocysteine in peripheral blood obtained from normal pregnant women and pregnant patients with preeclampsia at the Departments of Obstetrics and Gynecology of the Ghaem Hospitals in Mashhad University of Medical Sciences, Mashhad, Iran from May 2004 to August 2006. Preeclampsia patients were diagnosed as such when a blood pressure higher than 140/90 mm Hg was observed on at least 2 occasions more than 6 hours apart, after the 20th week of pregnancy, and a proteinuria higher than 300 mg/24 h was detected. Severe preeclampsia was diagnosed on the basis of diastolic blood pressure ≥110 mm Hg or significant proteinuria (dipstick measurement of ≥2+) or the presence of severity evidence such as headache, visual disturbances, upper abdominal pain, oliguria, convulsions, elevated serum creatinine, thrombocytopenia, marked liver enzyme elevation, and pulmonary edema. Inclusion criteria for the preeclamptic group were: primigravida who visited hospital during 28-40 weeks gestation. Exclusion criteria were the following: previous chronic hypertension, failure to stop folate supplementation (5 mg/day) by the 12th week of gestation, special diets implying a folate consumption higher or lower than normal intake in our geographical area (assessed by a nutritional questionnaire), altered renal function, diabetes or chronic diseases, treatment with antifolate drugs (antiepileptics, methotrexate), twin pregnancies, maternal age above 40 years old. All normal pregnant women were singleton primigravidas monitored at the Department of Obstetrics and Gynecology of our hospital with gestational age 28-40 weeks, no chronic medical disorders, and not in labor. They were normotensive and had normal blood pressures throughout gestation. All participants in this study signed an informed consent agreement. Our Hospital Ethics Committee approved the study. The study group consisted of 40 women with normal pregnancy, 37 women with mild preeclampsia, and 38 women with severe preeclampsia. Three groups were similar in age and body weight (mild preeclampsia group mean age: 27.4±6.4 years, severe preeclampsia: 26.1±5.8 and pregnant control group: 24.6± 4.2 years). Blood samples were collected through an indwelling ante-cubital venous catheter. None of the patients or control subjects were treated with any antihypertensive or other medications, and none of them were in active labor at the blood collection time. Collected blood samples were centrifuged at 1500 g for 10 minutes and stored at -20°C until assayed for homocysteine. In all patients, and normal pregnant women, serum homocysteine level was performed with enzyme linked immunosorbent assay (Axis-Shield Diagnostics, Dundee, UK). The lowest limit of detection was 1.0 µmol/L. The maximum inter-and intra-assay coefficients of variation for the range of concentrations evaluated were 8% for homocysteine.

The results were expressed as mean±SD and analyzed by an independent samples t-test. All statistical analyses were carried out using SPSS 11.0 software package from SPSS Inc., Chicago, USA. The level of significance was set at $p<0.05$.

Results. This study included 40 normal pregnant women and 75 pregnant women with pre-eclampsia (37 mild pre-eclampsia, and 38 severe pre-eclampsia). **Table 1** lists the clinical characteristics of the 3 study groups. There was no difference in the mean gestational age at venipuncture and the birth weight between normal pregnant and mild pre-eclamptic women. However, the mean gestational age at delivery ($p=0.0001$) and the birth weight ($p=0.05$) were significantly lower in the group with severe pre-eclampsia than in normal pregnant women. There was no statistical difference in bilirubin, creatinine, blood glucose, uric acid, hemoglobin, hematocrit, and platelets between mild and severe preeclampsia, while liver enzyme test and urine protein was significantly different between the 2 groups ($p<0.05$). Homocysteine was detected in all specimens. There was no significant difference in the mean homocysteine level between normal pregnant women and mild pre-eclamptic women. Patients with severe pre-eclampsia had significantly higher mean plasma levels than normal pregnant and mild pre-eclamptic women (**Table 1**).

Discussion. This case-control study demonstrates that women with severe preeclampsia have higher plasma homocysteine levels. There was a correlation between homocysteine concentration and severity of preeclampsia. There is strong evidence that homocysteine correlates with an increased risk of cardiovascular disease, stroke, and a range of other thromboembolic conditions, although it is not clear whether the association is causal or an effect of the disease process.[9,10] Plasma tHcy concentrations in women in the second and third trimesters of pregnancies without complications are lower than values found in non-pregnant women (median: 8.5, range: 5.8-12.8 mmol/l).[11] In our study, plasma tHcy

Table 1 - Characteristics of normotensive and preeclamptic pregnant women.

Characteristics	Uncomplicated pregnancy (n = 40)	Mild preeclampsia (n = 37)	Comparison between normal pregnant and mild preeclampsia	Severe preeclampsia (n = 38)	Comparison between women with mild and severe preeclampsia	Comparison between normal pregnant and women with severe preeclampsia
Maternal age (years) mean ± SD	24.6 ± 4.2	27.4 ± 6.4	NS	26.1 ± 5.8	NS	NS
Gestational age (weeks) mean ± SD	37.1 ± 2	35.7 ± 4	0.001	32.7 ± 5.6	NS	0.0001
Maternal weight mean ± SD	71.4 ± 10.4	77 ± 12.5	NS	71.1 ± 11.4	NS	NS
Fetal weight (kg) mean ± SD	2.6 ± 0.7	2.3 ± 0.68	NS	2.1 ± 0.97	NS	<0.05
Blood pressure (mm Hg)						
Systolic	111 ± 14	149.1 ± 15	<0.001	154.7 ± 19.7	NS	<0.001
Diastolic	63 ± 12	92 ± 12	<0.001	107.6 ± 14.8	<0.001	<0.001
Homocysteine	8.8 ± 2.8	10.4 ± 2.3	NS	13.8 ± 7/0	<0.05	<0.05

NS - not significant, $p<0.005$ was considered statistically significant

concentrations in the control group was similar to other studies. Plasma tHcy decreases during pregnancy, probably due to increased plasma volume, which is responsible for hemodilution, hormonal changes associated with pregnancy, and increased fetal need for methionine. The stand up of homocysteine to fetal metabolism is demonstrated in several studies.[12] There has been reported to be an association between hyperhomocysteinemia and preeclampsia,[12-16] although there is some controversy on this point.[17-19] In this study, hyperhomocysteinemia in women with preeclampsia was observed similarly as previous reports.[20] In many reports, plasma homocysteine concentration was studied in patients with preeclampsia without showing any attention to the severity of preeclampsia. The results of this study showed that homocysteine concentration in severe preeclamptic was significantly higher than mild preeclampsia and normotensive women. On the contrary, Gurbuz et al[21] found that homocysteine concentration in mild preeclampsia was higher than that in the control.[21] Conversely, Middeldrop et al[19] reported the concentration was not related to the risk of preeclampsia, instead, they claimed that women with hyperhomocysteinemia tended to have a lower risk for preeclampsia.[19] This claim is contrary to other reports.[22,23] Another reported study showed the serum homocysteine concentration in patients with preeclampsia was higher than those with uncomplicated pregnancy,[22] which is similar to our study. They also reported there was no dose-response relationship between hyperhomocysteinemia and preeclampsia. Homocysteine concentration in preeclamptic pregnant women reported in 25 articles was assessed, however, severity of preeclampsia was determined only in 4 studies, and 2 of these studies measured homocysteine concentration after clinical onset of preeclampsia. Ingec et al[24] demonstrated a relationship between increased homocysteine and severity of preeclampsia. As the levels of plasma homocysteine were not different between patients with mild preeclampsia and normotensive pregnancy, the increased homocysteine concentrations were thought to be the result of severity of preeclampsia.[24] Homocysteine may produce oxidative stress and endothelial cell dysfunction. In our study, the concentration of homocysteine was not changed in mild preeclampsia. This result may be due to minimally effected endothelial dysfunction by low levels of homocysteine. The elevated concentration of homocysteine may indicate the patient with preeclampsia progressing to a severe form.

In conclusion, our results suggest that an increased level of plasma homocysteine is associated with clinical severity of preeclampsia. It requires further study in which the severity of preeclampsia will be classified to investigate the relationship between plasma homocysteine concentration and severity of preeclampsia.

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