Elevated Plasma Homocysteine Concentrations in Severe Preeclampsia and Eclampsia

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INGEC, M., BOREKCI, B. and KADANALI, S. Elevated Plasma Homocysteine Concentrations in Severe Preeclampsia and Eclampsia. Tohoku J. Exp. Med., 2005, 206 (3), 225-231 — Homocysteine is an essential amino acid required for the growth of cells and tissues in the human body. Maternal hyperhomocysteinemia is associated with a number of placenta-mediated diseases such as preeclampsia. The aim of this study was to evaluate the plasma level of homocysteine and its association with severity of preeclampsia. A case-control study was performed with 32 mild preeclamptic patients, 25 severe preeclamptic patients, 16 eclamptic patients and 34 controls. Maternal plasma homocysteine concentration was measured prospectively at antenatal period by high-performance liquid chromatography. There were no significant differences in demographic characteristics between the study and control groups. Mean plasma levels of homocysteine in women with severe preeclampsia (16.7 ± 10.1 μmol/l, mean ± s.d., n = 25) and eclampsia (16.5 ± 9.6 μmol/l, mean ± s.d., n = 16) were significantly higher than those in mild preeclampsia (7.7 ± 2.4 μmol/l, mean ± s.d., n = 32) and controls (6.7 ± 1.6 μmol/l, mean ± s.d., n = 34) (p < 0.0001). It should be noted that plasma levels of homocysteine are not significantly different between mild preeclampsia and controls. In conclusion, plasma homocysteine concentrations are increased in severe preeclampsia and eclampsia, but not in mild preeclampsia.

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Homocysteine is a sulfur containing amino acid primarily derived from demethylation of dietary methionine, which is abundant in proteins of animal origin. It is an essential amino acid required for the growth of cells and tissues in the human body. Elevated circulating homocysteine is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis and occlusive vascular disorders (Refsum et al. 1998). The vascular effects of hyperhomocysteinemia have been proposed to include endothelial cell injury and thrombus formation (Welch and Loscalzo 1998). Levels of maternal serum homocysteine normally decrease with gestation, either due to a physiological response to the pregnancy, increase in estrogen, hemodilution from increased plasma volume, or increased demand for methionine by both the mother and fetus (Walker et al. 1999). Maternal hyperhomocysteinemia has been associated with a number of placenta-mediated diseases such as preeclampsia (Ray and Laskin 1999).

Although the exact incidence of preeclampsia is unknown, it has been reported to be approximately 5-8% (ACOG Practice Bulletin 2002). If
preeclampsia is not diagnosed or treated, it can progress to maternal multiorgan failure, coagulopathy, and maternal and fetal death in its severe form (Friedman et al. 1991; Barron 1992). Its pathophysiologic process is poorly understood, but currently, endothelial dysfunction is most popularly hypothesized to be a central pathophysiologic feature of preeclampsia, leading to altered vascular reactivity, loss of vascular integrity and activation of the coagulation cascade (Roberts 1998).

The homocysteine-mediated vascular changes are similar to those associated with preeclampsia, therefore, a hypothesis has been proposed that hyperhomocysteinemia may be associated with this condition (Rajkovic et al. 1997). Several studies have indicated that homocysteine concentrations are increased in women with preeclampsia (Zeeman et al. 2003). But, there are a few reports concerning hyperhomocysteinemia in patients with eclampsia (Rajkovic et al. 1999; Gurbuz et al. 2004). We therefore planned this study to investigate plasma levels of homocysteine in women with eclampsia, and to assess whether there is an association between hyperhomocysteinemia and the severity of preeclampsia.

**MATERIALS AND METHODS**

This case-control study was conducted at the Ataturk University, Medical Faculty Hospital, in Erzurum, from January 2004 through February 2005. It included 32 patients with mild preeclampsia (Group 1), 25 patients with severe preeclampsia (Group 2) and 16 patients with eclampsia (Group 3). The control group was matched with study groups for age, weight and gestational age during study, and consisted of 34 normotensive healthy pregnant women (Group 4). The ethics committee of Ataturk University, Medical Faculty, has approved this study.

Mild preeclampsia was defined as a systolic blood pressure (BP) 140 mmHg or greater or a diastolic BP of 90 mmHg confirmed by 6 or more hours apart, where as proteinuria was defined as 300 mg or more protein in a 24-hour urine collection or +1/+2 proteinuria on dipstick urinalysis. Severe preeclampsia was defined if diastolic BP increased up to at least 110 mmHg or systolic BP 160 mmHg or higher, along with proteinuria 3+ on dipstick, or at least 5,000 mg/day, and presence of headache, visual disturbances, upper abdominal pain, oliguria (< 500 ml/24 h), hyperbilirubinemia, elevated serum creatinine levels (> 0.8 mg/100 ml), thrombocytopenia (< 150,000/mm³), elevated aspartate or alanine transcarbamylase levels, and marked fetal growth restriction (ACOG Practice Bulletin 2002). Eclampsia was defined as the onset of convulsions or coma in a patient who has signs and symptoms of preeclampsia.

Exclusion criteria were the patients with pre-existing hypertensive, cardiovascular or renal disease, diabetes mellitus or chronic disease, multiple pregnancies, treatment with antifolate drugs (antiepileptics, methotrexate).

We have collected venous blood samples from patients’ antecubital vein between 07:00 - 09:00 after an overnight of fasting. This was not convenient in a few numbers of eclamptic patients due to their clinical situation. These samples were centrifuged for 10 min at 2,500 rpm and plasma was separated and stored at −40°C until analysis. The plasma homocysteine levels were measured by high performance liquid chromatography method (ClinRep, HPLC, Munich, Germany). The analytical sensitivity of the assay was 0.5 μmol/l, and the intraassay and interassay coefficients of variation were 3.9% and 5.8%, respectively.

Statistical analysis was performed using SPSS (version 10.0 for windows) software. Categorical variables were analyzed using the χ² test. Kruskall-Wallis test for the analysis of variance was used when all groups were compared for continuous variables. When significant differences were detected between the groups, the results were rearranged with Bonferroni-Dunn post hoc method and then the groups were compared for statistical significance using Mann Whitney’s U-test. Correlation coefficients were evaluated using linear regression analysis. P < 0.05 was considered statistically significant.

**RESULTS**

There were no significant differences in age, gestational age, body mass index (BMI) and the rate of primiparous between the study and control groups. As expected, the mean arterial pressure (MAP) was higher in all study groups than control, and birth weight was higher in controls than study groups (p < 0.0001) (Table 1).

The mean levels of plasma homocysteine of the three study groups and control patients are shown in Fig. 1. The plasma levels of homocysteine were significantly higher among women
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Table 1. Characteristics of the study and control groups

<table>
<thead>
<tr>
<th></th>
<th>Mild PE</th>
<th>Severe PE</th>
<th>Eclampsia</th>
<th>Control</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>32</td>
<td>25</td>
<td>16</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>25.2 ± 3.4</td>
<td>24.8 ± 3.4</td>
<td>22.7 ± 3.0</td>
<td>26.4 ± 5.4</td>
<td>0.07</td>
</tr>
<tr>
<td>GA at study (wk)</td>
<td>35.2 ± 2.7</td>
<td>34.1 ± 2.7</td>
<td>34.9 ± 1.9</td>
<td>35.3 ± 3.3</td>
<td>0.051</td>
</tr>
<tr>
<td>GA at delivery (wk)</td>
<td>37.0 ± 1.7</td>
<td>34.7 ± 2.0</td>
<td>34.9 ± 1.9</td>
<td>38.2 ± 1.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.8 ± 2.3</td>
<td>27.6 ± 2.0</td>
<td>28.3 ± 1.7</td>
<td>27.7 ± 1.2</td>
<td>0.83</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>113.8 ± 4.1</td>
<td>122.0 ± 14.5</td>
<td>125.0 ± 6.0</td>
<td>78.5 ± 6.7</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Primiparous (%)</td>
<td>34.4</td>
<td>28.0</td>
<td>50.0</td>
<td>29.4</td>
<td>0.46</td>
</tr>
<tr>
<td>Hemoglobin (g/100 ml)</td>
<td>11.7 ± 1.6</td>
<td>12.9 ± 2.2</td>
<td>12.2 ± 2.0</td>
<td>12.5 ± 2.3</td>
<td>0.13</td>
</tr>
<tr>
<td>Platelet (mm(^3))</td>
<td>202,000 ± 47,000</td>
<td>161,000 ± 69,000</td>
<td>162,000 ± 78,000</td>
<td>253,000 ± 54,000</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>31.8 ± 12.4</td>
<td>90.2 ± 137.6</td>
<td>127.1 ± 125.2</td>
<td>24.7 ± 8.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>20.3 ± 8.6</td>
<td>69.3 ± 118.4</td>
<td>79.0 ± 110.6</td>
<td>18.9 ± 8.0</td>
<td>0.03</td>
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<tr>
<td>LDH (U/l)</td>
<td>639 ± 201</td>
<td>1,118 ± 371</td>
<td>1,668 ± 1,529</td>
<td>525 ± 101</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Creatinine (mg/100 ml)</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.5</td>
<td>0.9 ± 0.4</td>
<td>0.7 ± 0.3</td>
<td>0.48</td>
</tr>
<tr>
<td>Uric acid (mg/100 ml)</td>
<td>5.9 ± 1.9</td>
<td>7.3 ± 2.2</td>
<td>8.8 ± 1.8</td>
<td>3.7 ± 0.6</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Proteinuria (mg/d)</td>
<td>1,550 ± 1,080</td>
<td>4,740 ± 1,550</td>
<td>5,400 ± 1,890</td>
<td>177 ± 70</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2,859 ± 490</td>
<td>2,346 ± 590</td>
<td>2,275 ± 735</td>
<td>3,013 ± 530</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

PE, preeclampsia; GA, gestational age; MAP, mean arterial pressure; BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase.

Fig. 1. A simple boxplot comparing plasma homocysteine concentrations in study and control groups. Boxes represent 50% of the samples; bars represent 95% of the samples. The horizontal bar within each box illustrates the mean value calculated for each group.

Fig. 2. Relationship between plasma homocysteine concentration and mean arterial pressure (MAP). Significant positive correlation is seen between plasma concentrations of homocysteine and mean arterial pressure (\( r = 0.435, p < 0.0001 \)).
with severe preeclampsia (16.7 ± 10.1 μmol/l, mean ± s.d., n = 25) and eclampsia (16.5 ± 9.6 μmol/l, mean ± s.d., n = 16) than mild preeclampsia (7.7 ± 2.4 μmol/l, mean ± s.d., n = 32) and controls (6.7 ± 1.6 μmol/l, mean ± s.d., n = 34) (p < 0.0001). However, plasma levels of homocysteine were not different between mild pre-eclampsia and controls. No significant difference was detected in the serum levels of hemoglobin and creatinine among all groups. The levels of alanine aminotransferase (ALT) (p = 0.03), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), uric acid and proteinuria in study groups were higher (p < 0.0001), but platelet

Fig. 3. Relationship between plasma homocysteine concentration and aspartate aminotransferase (AST). Significant positive correlation is seen between plasma concentrations of homocysteine and AST (r = 0.687, p < 0.0001).

Fig. 4. Relationship between plasma homocysteine concentration and uric acid. Significant positive correlation is seen between plasma concentrations of homocysteine and uric acid (r = 0.217, p = 0.02)

Fig. 5. Relationship between plasma homocysteine concentration and proteinuria. Significant positive correlation is seen between plasma concentrations of homocysteine and proteinuria (r = 0.586, p < 0.0001)

Fig. 6. Relationship between plasma homocysteine concentration and platelet count. Significant negative correlation is seen between plasma concentrations of homocysteine and platelet count (r = −0.431, p < 0.0001).
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count was lower than control group ($p < 0.0001$). Significant positive correlations were observed between plasma concentrations of homocysteine and MAP (Fig. 2; $r = 0.435$, $p < 0.0001$), plasma concentrations of homocysteine and AST (Fig. 3; $r = 0.687$, $p < 0.0001$), plasma concentrations of homocysteine and uric acid (Fig. 4; $r = 0.217$, $p = 0.02$) and plasma concentrations of homocysteine and proteinuria (Fig. 5; $r = 0.586$, $p < 0.0001$).

But, significant negative correlation was observed between plasma concentrations of homocysteine and platelet count (Fig. 6; $r = -0.431$, $p < 0.0001$).

**DISCUSSION**

Elevated plasma level of homocysteine has been identified as an independent risk factor for atherosclerosis and occlusive vascular disease (Fallest-Strobl et al. 1997). Endothelial cell activation or dysfunction is the most popularly hypothesized factors for preeclampsia. Therefore, hyperhomocysteinemia during pregnancy may contribute to this condition. Plasma homocysteine concentration is reduced in normal pregnancy, probably due to increased plasma volume and associated hemodilution, increased glomerular filtration rate, hormonal changed associated with pregnancy and increased uptake of homocysteine by the fetus (Malinow et al. 1998; Powers et al. 1998; Hague 2003). Many studies have demonstrated the relationship between hyperhomocysteinemia and preeclampsia (Refsum et al. 1998; Walker et al. 1999; Hogg et al. 2000; Vollset et al. 2000; Lopez-Quesada et al. 2003), while others have refuted an association (Hietala et al. 2001; Rajijmakers et al. 2001; Middeldorp et al. 2004). This relationship has been shown in early pregnancy (Cotter et al. 2001), in the second trimester (Sorensen et al. 1999; Hogg et al. 2000), and in the third trimester of pregnancy (Sanchez et al. 2001). However, it has been suggested that midtrimester plasma homocysteine concentrations in asymptomatic women are not predictive of the subsequent development of preeclampsia (Hietala et al. 2001; Rajijmakers et al. 2001; D’Anna et al. 2004; Yu et al. 2004).

In this study, hyperhomocysteinemia in women with preeclampsia was observed similarly as previous reports (Rajkovic et al. 1997; Laivuori et al. 1999; Sanchez et al. 2001). In many reports, plasma homocysteine concentrations were studied in patients with preeclampsia without showing any attention to the severity of preeclampsia. In addition, there were just a few reports concerning the levels of plasma homocysteine in patients with eclampsia (Rajkovic et al. 1999; Gurbuz et al. 2004). Rajkovic et al. (1999) evaluated postpartum plasma homocysteine concentrations and found that it was higher among women with eclampsia and preeclampsia compared with normotensive women. But, the present case-control study is different from Rajkovic’s report studied in postpartum eclamptic patients, because of plasma levels of homocysteine in patients with eclampsia and mild preeclampsia were investigated at antenatal period in our study. The results of this study showed that homocysteine concentrations in severe preeclamptic and eclamptic women were significantly higher than mild preeclamptic and normotensive women. On the contrary, Gurbuz et al. (2004) found that homocysteine concentrations in mild preeclampsia were higher than that in the control and the levels in eclampsia were higher than that in severe preeclampsia. This difference may be due to study design. In the brief communication report of Gurbuz et al. (2004), the group with severe preeclampsia was divided into two subgroups: eclamptic women and women with HELLP syndrome. In that study, severe preeclamptic group consisted of 16 women with HELLP syndrome and cases of HELLP syndrome occurs 65% of severe preeclamptic group. The difference in homocysteine levels between severe and eclampsia cases are due to HELLP syndrome cases. In contrast, severe preeclamptic group does not include any cases of HELLP syndrome in our study. Severe preeclampsia was diagnosed according to the criteria reported ACOG Committee (ACOG practice bulletin 2002) in our study.

On the other hand, Middeldorp et al. (2004) 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. This claim is
contrary to many literatures (Rajkovic et al. 1997; Zeeman et al. 2003; Mignini et al. 2005). A currently reported study showed that serum homocysteine concentrations in patients with preeclampsia were higher than those with uncomplicated pregnancy (Mignini et al. 2005), which is similar to ours. They also reported there was no dose-response relationship between hyperhomocysteinemia and preeclampsia, because of a lack of consistency in the data. Homocysteine concentrations in preeclamptic pregnant women reported in 25 articles were assessed, but severity of preeclampsia was determined only in 4 studies, and 2 of these studies measured homocysteine concentrations after clinical onset of preeclampsia. The data of eclamptic patients were obtained only in one study. It needs more study in which severity of preeclampsia will be classified to investigate the relationship between plasma homocysteine concentration and the severity of preeclampsia.

An elevated plasma homocysteine level is associated with a variety of vascular disorders, but it is not clear whether the association is causal or an effect of the disease process. A meta-analysis demonstrated a relationship between homocysteine and coronary artery disease, cerebrovascular disease, and peripheral vascular disease (Boushey et al. 1995). The risk of coronary artery disease is seen to increase across a range of homocysteine values. Wald et al. (1998) suggested that it was a positive association between homocysteine and ischemic heart disease, for increase in homocysteine of 5-μmol/l, the rate of ischemic heart disease risk increased by 84%. Similarly, this study 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, this increased homocysteine concentrations were thought to be results of severity of preeclampsia. Homocysteine may produce oxidative stress and endothelial cell dysfunction (Roberts et al. 1991; Powers et al. 1998). 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 concentrations of homocysteine may indicate the patient with preeclampsia progressing to a severe form, but not predict eclamptic seizure.

This study has shown an increased plasma homocysteine concentration in severe preeclampsia and eclampsia, but not in mild preeclampsia.

References


levels of factor VIII: C or homocysteine are not at increased risk for obstetric complications. *Thromb. Haemost.*, 92, 787-790.


