Role of hyperhomocysteinaemia and folate deficiency in the development of preeclampsia and gestational hypertension
Saha S, Jahan S, Shakya NB

Abstract
Elevated homocysteine is an important biochemical marker of endothelial damage, which is known to be associated with preeclampsia (PE) and gestational hypertension (GH); however, as with other markers, the causal relationship between homocysteine and the disorders has not yet been established. The aim of the present study was to explore the causal relationship of homocysteine with PE and GH using a model, which analyses the plasma level of the molecules in early pregnancy. It was also explored whether folate, a known precursor in the homocysteine metabolism, play role in the postulated abnormalities of homocysteine in PE and GH. Hypertension was diagnosed by the criteria of American College of Obstetrics and Gynecology (ACOG). Among 226 pregnant women who were followed until the end of pregnancies, 7 developed preeclampsia and 17 developed GH. A group of 69 randomly selected normotensive subjects were taken as control. Subjects were investigated for their plasma levels of homocysteine and folate. All the study groups age and gestational week matched. Plasma homocysteine (mean±SD) in controls was 5.80±1.80, in PE: 5.5±0.85 (p=0.675) and in GH 5.82±1.24 (p=0.215). No significant differences in plasma homocysteine value in early pregnancy were found between the groups who developed PE or GH and who remained normotensive throughout the pregnancy. The odds ratio (OR) of subsequent PE with a value greater than 6.3mmol/l was 0.44, CI (0.11, 1.81, NS). The folate levels also did not differ between any 2 of the 3 groups. A significantly higher level of urinary protein-creatinine ratio was found in both PE and GH groups as compared to the control. Plasma homocysteine and folate may not have a predictive significance in the development of PE and GH. Early measurement of urinary protein-creatinine ratio in pregnancy may have a role in the prediction of PE and GH at the later stages of pregnancy.

Introduction
Hypertension, a common disorder in pregnancy, constitutes a major risk factor for morbidity & mortality for both mother and child all over the world. Hypertension complicates about 5-10% of pregnancies and includes gestational hypertension, chronic hypertension, PE, Eclampsia as per definitions from the National High Blood Pressure Education Program. Gestational hypertension may be an early sign of either PE or chronic hypertension.\(^1\) Preeclampsia is hypertension; associated with proteinuria occurring after 20\(^{th}\) weeks of gestation, which occurs most frequently near term (Mabie). Mortality from hypertensive disorder is much higher in the developing countries which is about 70-100 per 100,000 live births.\(^2\) The incidence of PE in the developed countries has been reduced to 0.02% to 0.05% of all deliveries with a fatality of 2%.\(^3\) PE and GH are highly important public health problems. Available epidemiological evidences support the view that, PE is a disease of multiple theories. Among them genetics, immunologic, circulatory, uterine vascular changes and endothelial dysfunction is important. Current hypothesis for the pathogenesis of PE states immunological disturbances includes

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abnormal placentation resulting in decreased placental perfusion and release of various circulatory factors from placenta to both maternal and fetal circulation. These may lead to endothelial cell injury and vascular pathology with changes in vasomotor tone and coagulation. Studies show the vascular endothelial damage and dysfunction is present in uteroplacental bed in PE. PE is a systemic disorder that occurs in the presence of placenta. Since delivery of the placenta abates the problem. Thy placenta is thought to be the key to its pathogenesis. Homocysteine is a sulphhydryl containing amino acid derived from the metabolic demethylation of dietary methionine, which is abundant in animal protein. It is present in plasma in four forms. The term total plasma (or serum) homocysteine (tHcy) refers to the combined pool of all four forms of homocysteine. An abnormal tHcy is defined by an arbitrary cut off in the distribution of concentrations found in the normal population in much the same way as hypertension and hypercholesterolaemia have been defined. Several factors increase plasma homocysteine level like genetic defects in homocysteine metabolism, nutritional deficiencies in vitamin cofactors, increasing age, male sex, menopause and several diseases. Homocysteine levels decrease significantly during pregnancy and are lowest in the second trimester. The fall in homocysteine parallel the decrease in albumin with pregnancy progression. This finding is not expected because 70% to 80% of homocysteine is albumin bound. Another mechanism responsible for the reduction in homocysteine levels during pregnancy is utilization by the fetus. There is a decreasing plasma homocysteine concentration gradient from the maternal via a umbilical vein to umbilical artery, obtained at delivery, suggesting incorporation of homocysteine in the fetal metabolic cycle. The most common enzyme defect associated with moderately raised tHcy is a point mutation in the coding region of the gene for Methylene tetrahydrofolate reductase (MTHFR), which is associated with a thermo labile MTHFR variant that has about half normal activity.

Patients and methods
The study was carried out for a period of one year in the OPD of Gynaecology and Obstetrics department, Bangabandhu Sheikh Mujib Medical University (BSMMU). The prospective study was taking pregnant women of early trimester up to delivery. The study population comprised of pregnant women from urban or semi urban or rural areas who attended the antenatal check up of the study place. Single on pregnancies of early trimester with no associated medical complaining were included for research purpose. A total number of 330 patients were taken for the study. Finally, after fulfilling all criteria, 281 patients were enrolled for final assessment and during follow up, 226 patients were continued. A questionnaire was developed to obtained relevant information of demographic and socio-economic data. Anthropometric data included weight, height and body mass index and blood pressure were measured. Subjects were requested to fast over night (12 hours) and not to smoke or take any kind of medicine on the previous day. Blood samples were collected and relevant investigations were done with plasma to exclude diabetes mellitus, renal disease and hyperlipidaemia. The plasma samples were analyzed for fasting glucose, TG, total cholesterol, HDL, LDK, creatinine, folate and homocysteine. Plasma homocysteine concentrations were measured by florescence polarization immunoassay (FPIA) technology and Plasma folate level estimation by competitive immunoassay technique using IMMULITE analyzer. Data are expressed as mean+_SD for parametric values and median for nonparametric values. The relationship between homocysteine and other variables was examined using Spearmen's nonparametric coefficient correlation analysis.

Results
In Table 1, total 281 pregnant women were included in the study. Among them 65
patients were lost in the follow up, 7 pregnant women developed preeclampsia and 17 developed GH among the remaining 226 patients. Thus the proportion of PE was 3.09% and GH 7.52%.

Table 1: Proportion of PE and GH cases

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of case</th>
<th>Percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH</td>
<td>17</td>
<td>7.52</td>
</tr>
<tr>
<td>PE</td>
<td>7</td>
<td>3.09</td>
</tr>
<tr>
<td>Control</td>
<td>202</td>
<td>89.38</td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
<td>100.00</td>
</tr>
</tbody>
</table>

In Table 2, the plasma homocysteine, mean±SD of the study groups were 5.50±0.85 as control, 5.80±1.80 as PE and 5.82±1.24 as GH. There was no significant difference in Plasma homocysteine levels between different groups.

Table 2: Homocysteine status of the study subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma Homocysteine (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control  (n=69)</td>
<td>5.50±0.85</td>
</tr>
<tr>
<td>PE (n=7)</td>
<td>5.80±1.80</td>
</tr>
<tr>
<td>GH (n=17)</td>
<td>5.82±1.24</td>
</tr>
</tbody>
</table>

In Table 3, the plasma folate, mean±SD of the study groups were 10.92±4.35 as control, 13.42±5.58 as PE and 10.04±5.21 as GH. There was no significant difference in folate (n mol/l) levels between different groups.

Table 3: Plasma folate status of the study subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma Folate (n mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control  (n=69)</td>
<td>10.92±4.35</td>
</tr>
<tr>
<td>PE (n=7)</td>
<td>13.42±5.58</td>
</tr>
<tr>
<td>GH (n=17)</td>
<td>10.04±5.21</td>
</tr>
</tbody>
</table>

Figure 1 showed homocysteine related to case and control study. Figure 2 and 3 showed that relationship between Folate and Homocysteine in the PE and GH subjects and relationship between Folate and Homocysteine in the control subjects respectively.

Discussion

Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality. In the present study the proportion of the PE cases were 3.09%, which is similar to worldwide incidence of preeclampsia. The proportions of
GH cases were 7.52%, which is similar to incidence found by Gilford et al.\(^1\) Elevated concentrations of homocysteine have been associated with increased risk of vascular disease.\(^1\) In the present study, plasma tHcy level ranges from 3.80-7.20 (mmol/l) with a mean value of 5.50 (mmol/l) in early pregnancy in uncomplicated pregnancy. This finding is consistent with previous findings by Danna et al.\(^2\) The present study is not able to demonstrate any difference in plasma homocysteine levels at early pregnancy between women who later developed PE or GH and those who remained normotensive. The plasma homocysteine, mean±SD of the study groups were 5.50±0.85 as control, 5.80±1.80 as PE and 5.82±1.24 as GH. Cotter et al carried out a prospective study taking 54,000 patients attending their 1st antenatal visit, mean±SD plasma homocysteine value of cases and controls were 9.8±3.3mmol/l and 8.4±1.9 mmol/l respectively with a p value of <0.0001.\(^3\) In the present study plasma folate ranges between 2.22-19.62 n mol/l with a mean±SD in 10.92±4.35 as control, 13.42±5.58 as PE and 10.04±5.21 as GH. There may be two reasons for the observed increase of folate concentration in the present study, one may be dietary intake; another may be folate supplementation during pregnancy. There was no correlation between homocysteine and folate in PE, GH and control group. However, when PE and GH group were combined, there was significant negative correlation between homocysteine and folate as expected.

**Conclusion**

Preeclampsia and gestational hypertension are two hypertensive disorders of pregnancy with involvement of both placental and maternal circulation. Elevated homocysteine is an important biochemical marker of endothelial damage, which is known to be associated with PE and GH; however, as with other markers, the causal relationship between homocysteine and the disorders has not yet been established.

**References**

Original Article
