Association of Maternal Serum Homocysteine Levels with Pre-eclampsia

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Abstract

Background: Pre-eclampsia is a major cause of maternal or fetal morbidity and mortality. Its pathophysiology is still an area of dilemma. Homocysteine is a sulfur containing amino acid, which damages smooth muscle of the vessel wall. The objective of this study was to investigate the association of serum homocysteine levels with preeclampsia.

Materials and methods: This case control study was carried out in the Department of Obstetrics & Gynecology Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from April 2017 to March 2018. The study reviewed a total of 108 women with pregnancy. These participants were categorized into three groups: healthy pregnant subjects group, mild preeclampsia group and severe preeclampsia group. The three study groups were statistically similar in aspects of maternal age, gestational age and parity.

Results: The mean serum homocysteine level was found 16.4±7.9 µmol/l in mild preeclampsia group, 20.8±8.0 µmol/l in severe preeclampsia group and 8.2±3.7 µmol/l in the group of normal pregnant women. Serum homocysteine was significantly increased in severe preeclampsia and mild preeclampsia in comparison to normal group without preeclampsia. Significant positive correlation was found between systolic blood pressure (p=0.001) diastolic blood pressure (p=0.001) with serum homocysteine level.

Conclusion: The study concluded that a significant positive association was found between pre-eclampsia and maternal serum homocysteine levels. This may provide informationonthe role of homocysteine in preeclampsia with a preventive strategy of consequences of this condition.

Key words: Hypertension; Maternal Homocysteine; Preeclampsia; Proteinuria.

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Introduction

Preeclampsia is one of the most common pregnancy associated disorders. It is a multiorgan disease of unknown etiology characterized by de novo development of hypertension and proteinuria after 20 weeks of gestation and sometimes progress into multiorgan cluster of varying clinical features. It may bedivided in mild and severe forms. The classification depends on the level of blood pressure elevation and the presence of symptoms or signs of end organ damage.[1]

Preeclampsia and related hypertensive disorders of pregnancy impact 5 to 8% of all births in the United States. Incidence rates for preeclampsia in the United States, Canada and Western Europe, range from 2-5%. In the developing world, severe forms of pre-eclampsia and eclampsia are more common, ranging as low as 4% to as high as 18% in parts of Africa.[2] Pre-eclampsia or eclampsia is decreasing in developed countries, but it is still a major cause of maternal death in developing countries like Bangladesh, where about 16% incidence of preeclampsia was reported.[3] It is due to lack of public awareness, inadequate health education, poor socio-economic condition and less antenatal coverage. It would be better to diagnose preeclampsia before clinical manifestation as prevention or delay in the onset of this disease would have a significant impact on maternal and perinatal outcome.

The risk of pre-eclampsia is 2 to 5 fold higher in pregnant women with maternal history of this disorder. Depending on ethnicity, the incidence of preeclampsia ranges from 3% to 7% in healthy nulliparous and 1% to 3% in multiparas.[4] Other

risk factors include age ≥35 years, medical history of chronic hypertension, kidney disease, diabetes, obesity, and pregnancy characteristics such as twin or molar pregnancy, previous history of preeclampsia, or fetal congenital abnormality.[5] The risk of preeclampsia and eclampsia is double among women of low socio-economic status.[6]

Preeclampsia may increase both fetal and maternal morbidity and mortality.[7] Complication related to preeclampsia include preterm birth, intrauterine fetal growth restriction, placental abruption, maternal pulmonary edema and eclampsia. The estimated incidence of eclampsia is 1 to 3 per 1000 preeclampsia patients.[8] The exact nature of the primary event causing preeclampsia is not known. However abnormal trophoblastic invasion of spiral arteries, inappropriate endothelial cell activation, exaggerated inflammatory response are key features in the pathogenesis of preeclampsia.[9] Current hypothesis demonstrates thatendothelial dysfunction is the central pathophysiological feature of preeclampsia, leading to altered vascular reactivity, loss of vascular integrity and activation of coagulation cascade.[10]

Homocysteine alters the effect of many clotting proteins on the endothelial cell surface leading to a prothrombotic environment. Thus, it is conceivable that hyperhomocysteinemia could affect placental function by promoting oxidative stress, thereby increasing contractile response and production of procoagulants along with vasoconstrictor and maternal uteroplacental perfusion via any of this mechanism.[11]

Homocysteine is a sulfur containing amino acid primarily derived from de-methylation of dietary methionine, which is abundant in protein of animal origin. It is an essential amino acid required for the growth of cells and tissues in the human body.[11] Levels of maternal serum homocysteine normally decrease with gestation, either due to physiological response to pregnancy, increase in estrogen, hemodilution from increase plasma volume or increased demand for methionine by both the mother and fetus[12].Homocysteine is metabolized via two main pathways: re-methylation to methionine or transulphuration to cystathionine and then to cystine. A defect in either leads to an accumulation of circulating homocysteine. The defect may be congenital, due to an inborn error of cystathionine-B-synthetase, or due to homozygosity for a C-T mutation of nucleotide 677 in the Methyle netetra Hydrofolate Reductase (MTHFR) gene.[13] Other reasons for hyper homocysteinaemia are nutrient related.Deficiencies of folate, vitamin B12 or vitamin B6 cause homocysteine to accumulate because re-methylation to methionine requires folate and vitamin B12 and transulphuration to cystathionine requires vitamin B6.[14]

Homocysteine damages smooth muscle of the vessel wall, creating a scratch inside the vessel where plaque can build up. Homocysteine is critically important during pregnancy. High maternal homocysteine levels increase the chance of miscarriage, preeclampsia or abruptio placenta. It can also lead to Intrauterine Growth Retardation, Low birth weight, prematurity and congenital malformations.[15] If an association between serum homocysteine concentration and preeclampsia is found, itmight be possible to identify pregnant women with risk of developing preeclampsia in earlier gestational age.Homocysteine level can be broughtback to normal range by vitamin supplementation reducing occurrence of preeclampsia can be avoided.[16]

Thus, this study could be used as a tool for prediction, diagnosis,treatment and follow-up of preeclampsia forbetter obstetric outcome, reducing maternal and neonatal morbidity and mortality.

Materials and methods

This antegrade case control study was conducted in the Department of Obstetrics and Gynecology, BSMMU, Dhaka from 1st April 2017 to 31st March 2018.Ethical clearance for the study was taken from the institutional review board, BSMMU. Permission for the study was taken from the concerned departments where this study was conducted. A total of 108 pregnant women, who attended antenatal clinic and admitted as indoor patientsof BSMMU at their 2nd half (20-40 weeks) of pregnancy were enrolled for the study. Recruited pregnant women were divided into case and control groups. Case groupconsisted of 72 pregnant women who were clinically diagnosed as preeclampsia.Among them, 36 were mild preeclampsia cases and 36 were severe preeclampsia cases during their second half of pregnancy. Control

group comprised of 36apparently healthy normotensive pregnant women in their second half of pregnancy (20 to 40 weeks).Age range of all study subjects werewithin 18 to35 years. Known casesof chronic hypertension,chronic renal disease, cardiovascular disease, diabetes mellitus,other chronic diseases and patients with treatment of antifolate drugs (antiepileptics, methotrexate) were excluded from the study.

Purposive sampling was done according to availability of the participants who had voluntarily joined this study. The purpose and procedure of study was discussed with the participants and informed written consent was taken. The study was anonymous and confidentiality of information was assured.An interviewer administered questionnaire was used for data collection.Detailed sociodemographic history, obstetric history, gestational age, family history and medical history were recorded in the pretested data sheet.Their antenatal records, early ultrasound scans were reviewed to confirm the duration of gestation.Routinephysical examination, anthropometric measurements (Height, weight) were taken and obstetric examination were conducted and recorded.

The subjects with blood pressure ≥140/90 mm of Hg on two occasions were evaluated for presence of urinary protein by dipstick method to establish diagnosis of preeclampsia.Proteinuria is defined as the urinary excretion of protein 300 mg/ 24 hours or a urine protein/ creatinine ratio of ≥ 0.3 or dipstick reading ≥1+ persistent (Equivalent to ≥ 30mg/dl).[17]

When proteinuria found ≥1+(≥30mg/dl) in collected urine sample then the diagnosis of preeclampsia was established and were selected as case.Mild preeclampsia is defined as the presence of hypertension BP > 140/90 mm Hg, but less than 160/110 mm Hg on 2 occasions, at least 6 hours apart without significant proteinuria. Severe preeclampsia is defined as the presence of having one or more of the following manifestations: systolic blood pressure more than 160 mm Hg and diastolic blood pressure more than 110mm Hg on 2 occasions, at least 6 hours apart with any of the following: proteinuria, oliguria (< 400 ml in 24 hours) cerebral or visual disturbance, epigastric or right upper quadrant pain, pulmonary oedema or cyanosis, impaired liver function, thrombocytopenia (Platelet count < 100000/ mm^3) and fetal growth restriction.

With all aseptic precaution 05 ml antecubital venous blood sample was collected from each subject for measurement of serum homocysteine. The blood sample was transferred into a clean, dry test tube and taken to the laboratory. Blood samples were centrifuged for 10 minutes at a rate of 4000 rmp. Serum homocysteine concentration was measured by Chemiluminescence Microparticles Immunoassay method in Abbott Architect system (ci8200). Whenever possible, the analysis was done immediately. If delayed, the samples were stored at -20^o Celsius till further analysis. The cut-off point of reaction was standardized by the manufacturer. samples with calculated reactivity value> 15µmol/l were considered positive.

Results

Table I : Distribution of the study patients according to age and Gestational age (n=108)

	Mild Preeclampsia (n=36)	Severe Preeclampsia (n=36)	Normal Pregnancy (n=36)	p value
Age (In years)	27.86±3.4	28.94±3	28.33±5.22	0.517^{ns}
Gestational age (Weeks)	31.78±3.61	32.92±3.16	33.58±3.86	0.099 ^{ns}

ns = not significant, p value reached from ANOVA test.

Table I shows age distribution of the study patients.The difference was statistically not significant (p>0.05) among three groups. This table also shows gestational age of the study subjects.The difference was also not statistically significant (p>0.05) among three groups.

Table II : Distribution of the study patients according to parity (n=108)

Parity	Mild Preeclampsia		Severe Preeclampsia		Normal Pregnancy		p value
Nulliparous	20	55.6	17	47.2	15	41.7	0.455^{ns}
Multiparous	16	44.4	19	52.8	21	58.3	

ns = not significant, p value derived from Chi square test.

Table II shows parity of the study patients, it was observed that 55.6% patients had nulliparous in mild preeclampsia, 47.2% in severe preeclampsia and 41.7% in normal pregnant group. The difference was statistically not significant (p>0.05) among three groups.

Figure 1 shows bar diagram showing serum homocysteine of the study patients and the difference was statistically significant (p<0.05) among three groups.

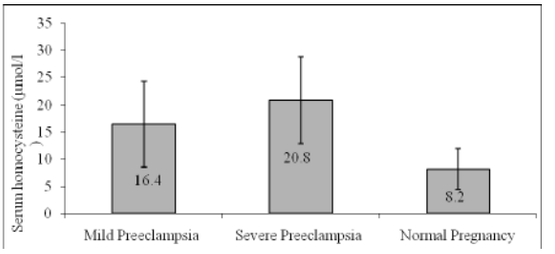

Fig 1 : Bar diagram showing mean serum homocysteine of the study patients

Table III : Relation of serum homocysteine levels with study population (n=108)

Serum homocysteine (µmol/l)	Pregnant mothers with Preeclampsia (n=72)		Normal Pregnancy (n=36)		p value
	n	%	n	%	
<5	6	8.3	2	5.6	0.001[s]
5-15	15	20.8	30	83.3	
>15	51	70.8	4	11.1	

s = significant, p value reached from Chi-square test.

Table III Serum homocysteine level in study patients, it was observed that 70.8% patients had serum homocysteine >15 µmol/l in pregnant mothers with preeclampsia, 11.1% in normal pregnant group. The difference was statistically significant ($p<0.05$).

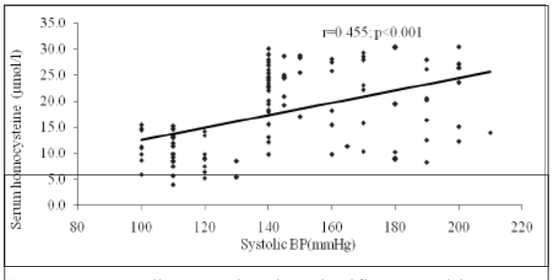

Fig 2 : Scatter diagram showing significant positive correlation (r=0.455, p=0.001) between systolic blood pressure with serum homocysteine level

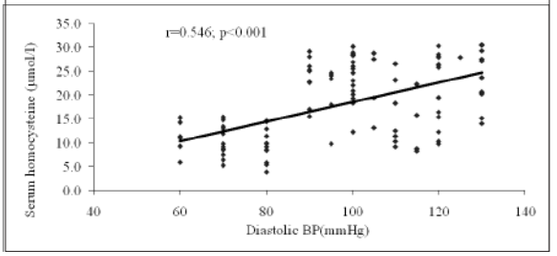

Fig 3 : Scatter diagram showing significant positive correlation (r=0.546, p=0.001) between diastolic blood pressure with serum homocysteine level

Discussion

Pre-eclampsia is a serious complication of pregnancy with unknown etiology that may occur at any stage of second or third trimester. Itis a leading cause of maternal and perinatal morbidity and mortality globally. Although the exact cause of pre-eclampsia is still unknown, it is known that in pre-eclampsia the basic pathology is endothelial dysfunction and intense vasospasm. Recently homocysteine, a metabolite of essential amino acid methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction. Elevated plasma homocysteine concentration is an independent risk factor for peripheral vascular diseases and for coronary artery diseases.[18] This case-control study was carried out with an aim to measure serum homocysteine levels in preeclampsia to find out its association with the occurrence and severity of preeclampsia. The findings of the present study were discussed and compared with previously published relevant studies.The present study consisted of 108 pregnant patients. These participants were divided into three groups: healthy group (n=36) mild pre-eclampsia (n=36) and severe pre-eclampsia (n=36). This study was conducted in the Department of Obstetrics and Gynecology, BSMMU.In this study, it was observed that the differencein mean age was statistically not significant among three groups. It was observed that 55.6% patients were nullipara in mild preeclampsia, 47.2% in severe preeclampsia and 41.7% in normal pregnant group. The difference was statistically not significant ($p>0.05$) among three groups. A study conducted by Yelikar and colleagues observed in their study that nulliparas were 50.0% in preeclampsia, 47.5% in eclampsia and 67.5% in controls.[18] Almost similar findings were also observed in some other studies.[19-21]

In the current study, it was observed that the mean serum homocysteine was 16.4±7.9 µmol/l in mild preeclampsia, 20.8±8.0 µmol/l in severe preeclampsia and 8.2±3.7 µmol/l in normal pregnant group.A study conducted by Yelikar and colleagues found the mean serum homocysteine levels in mild preeclampsia, severe preeclampsia was 14.99±3.47 µmol/l and 19.90±6.17 µmol/l and 12.48±2.95 µmol/l in normal pregnancy.[18] The study conducted by Ferdausi and colleagues found

the mean serum homocysteine levels in mild preeclampsia, severe preeclampsia was 10.43±5.12 mol/l and 11.5±4.58 mol/l and 5.70±1.30 mol/L in normal pregnancy.[16] In a similar study by Khosrowbeygi and colleagues found that women with severe preeclampsia had higher serum homocysteine than mild preeclampsia (17.40±2.7 vs 11.49±1.19 μmol/l) and normal pregnant women (17.40±2.7 and 6.38±0.3 μmol/l).[22] Wadhwani and colleagues, Kabra and colleagues, found in their studies that the serum homocysteine levels showed a highly significant increase with the severity of preeclampsia. In this present study, it was observed that 70.8% patients had serum homocysteine >15 μmol/l in pregnant mothers with preeclampsia, 11.1% in normal pregnancy.[20,23] The difference was statistically significant ($p<0.05$) between two groups. Makedos and colleagues compared homocysteine levels in a group of pregnant women with preeclampsia and control group free from preeclampsia and found the mean homocysteine level to be significantly increased in the former 11.11 μmol/L vs. 6.40 μmol/L, ($p<0.001$).[24] In this present study there is a significant positive correlation ($r=0.455$; $p=0.001$) was found between systolic blood pressure with serum homocysteine level.Othersimilar studies showed a positive correlation between serum homocysteine and systolic blood pressure.[16,19,25]. The above findings are closely resembled with the present study.In this present study significant positive correlation ($r=0.546$, $p=0.001$) was also found between diastolic blood pressure with serum homocysteine level, which is similar with some other studies.[16,25]

In addition, others report that elevated homocysteine levels in early pregnancy are known to be associated with the later development of mild PE.[26] A study suggests that elevations in homocysteine levels precede the clinical manifestation of preeclampsia by 8–16 weeks.[27] A number of other studies have also found higher levels of homocysteine in preeclampsia as compared with normal pregnant women.[28] Hasanzadeh and colleagues had also suggested that women with severe preeclampsia have higher homocysteine levels than women with mild preeclampsia or normotensive pregnant women.[29] The above investigators showed that serum homocysteine concentration in patients with PE were higher than those with uncomplicated pregnancy which were closely resembled with the present study. Therefore, the current study suggests that there is a clear evidence depicts the increasing homocysteine levels in severe preeclampsia and mild preeclampsia.

Limitations

The present study was conducted at a very short period of time. The study population was selected from one selected hospital. So the results of the study may not be reflect the exact picture of the country. Small sample size with purposive sampling was also a limitation of the present study. Therefore, in future further study may be under taken with large sample size. All the cases were preeclamptic before the measurement of homocysteine levels and so it cannot be determined whether the observed elevation in homocysteine preceded the development of preeclampsia.

Conclusion

This study was undertaken to investigate serum homocysteine levels and its association with the preeclampsia. Significant positive correlation was found between serum homocysteine levels with both systolic and diastolic blood pressure. This study revealed a relationship between serum homocysteine levels and severity of preeclampsia, where homocysteine levels significantly elevated in patients with preeclampsia compared with the control group. By using the serum homocysteine levels, we can diagnose the preeclampsia and its severity so we can intervene at the earliest. Adequate supplementation of Folic acid, B12, and B6 in preconceptional period will help to prevent hyper homocysteinemia and thus indirectly preeclampsia.

Recommendations

Our study suggests, Homocysteine is significantly associated with preeclampsia, its severity and have positive association with blood pressure. Further study with larger sample size in multiple centers may strengthen the outcome of this study result.

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Contribution of authors

BR - Conception, acquisition of data, drafting & final approval.

FZ - Design, interpretation of data, critical revision & final approval.

NH - Data analysis, critical revision & final approval.

NH - Acquisition of data, data analysis, drafting & final approval.

FI - Data analysis, interpretation of data, drafting & final approval.

KD - Acquisition of data, data analysis, critical revision & final approval.

SJ - Data analysis, drafting & final approval.

Disclosure

All the authors declared no competing interest.

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