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ORIGINAL ARTICLE

Serum Homocystine Level in Women with Severe Preeclampsia

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ABSTRACT

Objective: Though preeclampsia (PE) is an age-old disease, its pathology still remains obscure. Available epidemiological evidences suggest that PE is a disease of multiple theories. Recently serum homocystine level is considered as factor to be associated with preecampsia and the higher the level the greater is the severity of preeclampsia. The present study is aimed at investigating this hypothesized association.

Methodology: This case-control study was conducted over a period of 24 months from January 2012 to December 2013 in the Department of Obstetrics & Gynaecology, Dhaka Medical College, Dhaka. Pregnant women admitted with severe preeclampsia were the case, while pregnant women attending at the antenatal clinic without preeclampsia were the controls. Severe preeclampsia was diagnosed by blood pressure > 160/110 mm of Hg with proteinuria 3+ or more in dipstick test. The control group comprised of women of 24 - 40 weeks gestation, with blood pressure (both diastolic and systolic) remaining within normal limits without having any medical or obstetric complications. Data were analysed using software SPSS (Statistical Package for Social Sciences) version 16.0. The test statistics used to analyse the data were descriptive statistics, Chi-square (χ^2) Test, Student's t-Test and Receiver-operating characteristic (ROC) curve analysis.

Result: Around two-thirds of the patients in both groups were in the age range of 21-30 years with mean age of the cases and control being 25.8 ± 5.2 and 24.1 ± 3.7 years respectively (p = 0.108). Over three-quarters (77.5%) of the patients in case group and 60.0% in control group belonged to middle class (p = 0.091). Majorities of the cases (85.0%) and controls (90.0%) were preterm (gestational age < 37 weeks) with mean gestational age being 33.2 ± 3.3 and 32.3 ± 3.5 weeks in case and control groups respectively (p > 0.05). The patients in either group were predominantly primigravida. Seven (17.5%) patients in the case group gave the history of past preeclampsia as opposed to none in the control group (p = 0.005). Family history of preeclampsia was reported by the case group alone (p = 0.027). Majority (95.0%) of the cases had 3+ proteinuria. Serum homocystine concentration was significantly raised (15.7 ± 8.3 µmol/L) in case group than that in the control group (6.7 ± 1.3 µmol/L) (p < 0.001). Based on the receiver-operator characteristic (ROC) curve, serum homocysteine levels in pregnant women had the best area under the curve (0.975 or 97.5%) with sensitivity and specificity of the predictor variable being 92.5 and 77.5% respectively.

Conclusion: The study concluded that homocysteine levels are significantly elevated in patients with preeclampsia compared to the pregnant women without preeclampsia. Homocysteine may be of value in the monitoring of pregnancies to be complicated by preeclampsia.

Key words: Serum homocysteine, preeclampsia.

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INTRODUCTION:

Preeclampsia (PE) is a multisystem disorder of unknown etiology, unique to pregnancy with the onset after 20 weeks of gestation. It is considered severe if blood pressure and proteinurea are increased substantially when maternal multiorgan failure, coagulopathy and maternal and fetal death may occur.[1-3] It complicates 5-7% of all pregnancies;[4] 5-10% of which are severe.[5] In developing countries, PE complicates 4.4% of all deliveries and may be as high as 18% in some settings in Africa. In Bangladesh the incidence of PE ranges from 10 to 15% of all deliveries,[6] but only 2.3% of women end their pregnancy under medical supervision (whether it is abortion or delivery).[7] In a baseline survey of emergency obstetric care (EOC) situation in Bangladesh, 5% of total obstetrical admissions in health facilities were due to PE and Eclampsia.[7] As there are approximately 3.6 million births occur each year in Bangladesh, over 100,000 women develop eclampsia per year.[5]

The clinical condition was first discovered over 100 years ago, but still its pathology remains obscure. Available epidemiological evidences suggest that PE is a disease of multiple theories. Among them genetics, immunologic, circulatory, uterine vascular changes and endothelial dysfunction are important. Current hypothesis for the pathogenesis of PE states that immunological disturbance causes abnormal placentaion 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 that vascular endothelial damage and dysfunction is present in uteroplacental bed in PE.[8,9] Most recently homocysteine has been claimed as a factor for vascular endothelial cell injury in preeclampsia.[10] Homocysteine is a sulfur 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,[11] and is eliminated from the body via conversion into cystathion by a reaction catalyzed by vitamin B6 and Methionine catalyzed by vitamin B12 and folic acid as a cofactor.[12]

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 (e.g., 95th percentile) in the distribution of concentrations found in the 'normal population' in much the same way as hypertension and hypercholesterolaemia have been defined. Among fasting individuals 'normal' tHcy commonly ranges from 5 to 15 micro mol/L.[13] Homocysteine is found in low concentration in all tissues under normal condition.[14] The concentration of plasma homocysteine is regulated by several factors. These include genetically determined metabolic enzyme alteration, nutritional status (vitamin B12 and folate deficiency), underlying diseases, certain medications, age and pregnancy; of them, pregnancy is the only factor that specifically decreases the concentration of plasma homocysteine.[15]

Levels of maternal serum homocystetne 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 the fetus.[16] Elevated plasma homocysteine is, therefore, recognized as an important risk factors for preeclampsia and eclampsia.[17] There is strong evidence that homocysteine correlates with increased risk of cardiovascular disease, stroke and a range of other thromboembolic conditions and events including PE, although it is not clear whether the association is causal or a manifestation of the disease process. Causal relation between hyperhomocysteinemia and PE in Irish population had been observed by Cotter et al.[18] They concluded that elevated plasma homocysteine level in early pregnancy is associated with a 4-fold increased risk for development of non-severe PE and 3-fold increased risk of severe PE. Several other studies have also indicated that homocysteine concentrations are increased in women with preeclampsia.[19] But there are a few reports concerning hyperhomocysteinemia in patients with eclampsia.[20,21] Hyperhomocysteinemia is

treatable by supplementation of vitamins like vitamins B6, B12 and folic acid. So hyperhomocysteinemia, if detected in early pregnancy, taking supplementation might help substantially to reduce the occurrence of preeclampsia and adverse pregnancy outcome. The present study is, therefore, designed to investigate the serum homocysteine level and to evaluate its association with severity of preeclampsia in Bangladeshi population. The data obtained from the study may be useful in deciding whether plasma homocysteine as a screening test in early pregnancy could predict the development of PE and its severity.

MATERIALS AND METHODS:

This case-control study was conducted over a period of 24 months from January 2012 to December 2013 in the Department of Obstetrics & Gynaecology, Dhaka Medical College, Dhaka. Pregnant women admitted with severe preeclampsia, gestational age between 24 and 40 weeks were the case, while pregnant women (between 24-40 weeks) attending at the antenatal clinic without preeclampsia were the controls. Severe preeclampsia was diagnosed by blood pressure > 160/110 mm of Hg with proteinuria 3+ or more in dipstick test. The control group had both systolic and diastolic blood pressures within normal limits without any medical or obstetric complications. As 'normal' tHcy in fasting individuals commonly ranges from 5 to 15 micro mol/L, higher fasting values were classified arbitrarily as moderate (16-30 micro mol/L), intermediate (31-100 micromol/L), and severe (>100 micro mol/L) hyperhomocysteinaemia. Data were analysed using software SPSS (Statistical Package for Social Sciences) version 16.0. The test statistics used to analyse the data were descriptive statistics, Chi-square (χ^2) Test, Student's t-Test and Receiver-operating characteristic (ROC) curve analysis. The level of significance was set at 0.05 and p-value < 0.05 was considered significant.

RESULTS:

Age distribution shows that two-thirds of the patients in both groups were in the age range of 21-30 years with mean age of the patients in case and control groups being 25.8 ± 5.2 years and 24.1 ± 3.7 years respectively (p = 0.108). In terms of occupation, housewives formed the main bulk in both case and control groups (90.0% and 82.5% respectively). Over three-quarters (77.5%) of the patients in case group and 60.0% in control group belonged to middle class (p = 0.091) (Table I). Majorities of the cases (85.0%) and controls (90.0%) were preterm (gestational age < 37 weeks) with mean gestational age being 33.2 ± 3.3 and 32.3 ± 3.5 weeks in case and control groups respectively (p = 0.339).

TABLE I. Distribution of the study patients by their demographic characteristic (n= 80)

Demographic characteristic	Group		p-value
	Case (n = 40)	Control (n = 40)	
Age (in year)*			
≤20	8(20.0)	14(35.0)	
21-25	16(40.0)	20(50.0)	
26-30	12(30.0)	6(15.0)	
>30	4(10.0)	0(0.0)	
Mean ± SD#	25.8 ± 5.26	24.15 ± 3.69	0.108
Occupational status*			
Housewife	36(90.0)	33(82.5)	0.330
Job Holder	4(10.0)	7(17.5)	
Socioeconomic condition*			
Poor	9(22.5)	16(40.0)	
Middle Class	31(77.5)	24(60.0)	0.091
Rich	0(0.0)	0(0.0)	

Figures in the parentheses indicate corresponding %;
*__Chi-square Test__ (χ^2) was done to analyse the data.
Data were analyzed using __Unpaired t-Test__ and were presented as mean ± SD.

TABLE II. Distribution of the study patients by obstetric variables (n= 80)

Obstetric variables*	Group		p-value
	Case (n = 40)	Control (n = 40)	
Gestational age (weeks)			
< 37 (preterm)	34(85.0)	36(90.0)	0.339
≥ 37 (term)	6(15.0)	4(10.0)	
Gravida			
Primi	22(55.0)	27(67.5)	0.251
Multi	18(45.0)	13(32.5)	
History of past preeclampsia	7(17.5)	0(0.00)	0.005
History of preeclampsia in family	5(12.5)	0(0.00)	0.027

Figures in the parentheses indicate corresponding %;
*__Chi-square Test__ (χ^2) was done to analyse the data.

More than half (55%) of the patients in the case group and two-thirds (67.5%) in the control group were primigravida (p = 0.251). Seven (17.5%) patients in the case group gave the history of past preeclampsia as opposed to none in the control group (p = 0.005). Family history of preeclampsia was also solely reported by the case group (p = 0.027) (Table II). The mean systolic blood pressure was 169.0 ±10.3 mmHg and 115.0 ±17.0 mmHg in case and control respectively (p < 0.001), while the mean diastolic blood pressure was 115.1 ± 5.9 and 68.0 ± 11.0 mmHg respectively (p < 0.001) (Table III). Majority (95.0%) patients had 3+ proteinuria and 5.0% 2+ proteinuria (Fig 1). Serum homocystine concentration was significantly raised (15.7 ± 8.3 µmol/L) in case group than that in control group (6.7 ± 1.3 µmol/L) (p < 0.001) (Table IV). Of the 40 cases, 16(40%) had hyperhomocysteinaemia (serum homocystine > 15 mmol/L), while none of the control had raised serum homocystine.

TABLE III. Distribution of the study patients by blood pressure (n = 80)

Blood pressure #	Group		p-value
	Case (n = 40)	Control (n = 40)	
Systolic BP (mmHg)	169.0 ± 10.3	115.0 ± 17.0	0.001
Diastolic BP (mmHg)	115.13 ± 5.9	68.0 ±1.0	0.001

Data were analyzed using **Unpaired t-Test** and were presented as **mean ± SD.**

TABLE IV. Distribution of the study patients by serum homocysteine concentration (n= 80).

Serum homocysteine# concentration	Group		p-value
	Case (n = 40)	Control (n = 40)	
Serum Homocystine (µmol/L)	15.7 ± 8.3	6.7 ± 1.3	0.001
Range	7.46-38.2	4.06-8.39	

Figures in the parentheses indicate corresponding %;
Data were analyzed using **Unpaired t-Test** and were presented as **mean ± SD.**

TABLE V. Receiver-operator characteristic (ROC) curve of serum homocysteine levels for prediction of preeclampsia.

	Cut of value	Sensitivity	Specificity	Area under the ROC curve	95% Confidence interval (CI)	
					Lower bound	Upper bound
Serum homocysteine	8.0	92.5	77.5	0.975	0.947	1.003

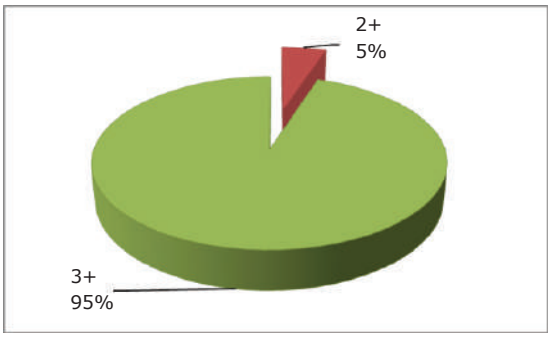

Fig 1: Distribution of the cases by urinary protein (n= 40)

Receiver-operator characteristic curve was constructed using serum homocysteine value of the participating women, which gives a cut off value of > 8.0 as the value with a best combination of sensitivity (92.5%) and specificity (77.5%) for the prediction of preeclampsia. Based on the ROC curve serum homocysteine levels had the best area under curve 0.975 or 97.5%. (Table V) (Fig 2).

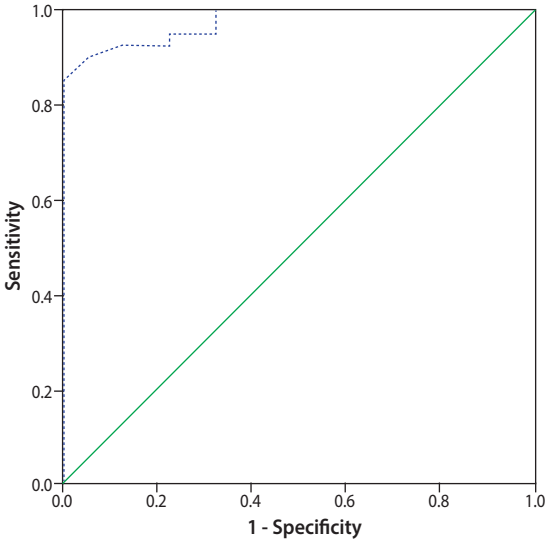

Figure 2: Receiver-operator characteristic curve of serum homocysteine levels.

DISCUSSION

In the present study the case and control groups were almost similar in terms of age distribution (25.8 ± 5.2 vs. 24.1 ± 3.7 years, p = 0.108). Majorities of the cases (85.0%) and controls (90.0%) were preterm (gestational age < 37 weeks) with mean gestational age being 33.2 ± 3.3 and 32.3 ± 3.5 weeks in case and control groups respectively (p > 0.05). Past history and

family history of preeclampsia were reported by few patients in the case group alone (p = 0.027). In this series most (95.0%) of the preeclamptic patients had severe proteinuria (3+).

Javadi et al.[22] showed the mean age of severe preeclampsia group and control group to be 27.7 ± 5.8 and 26.5 ± 5.8 years respectively, which is consistent with the current study. Similarly, Sangeeta et al.[23] mentioned that maximum number of the cases was in the age group of 21-25 years. In another study Franceschini et al.[24] reported that women developing preeclampsia were somewhat younger than the controls (29.8 ± 0.7 and 31.8 ± 0.5 years, p < 0.05). Thilaganathan et al.[25] showed mean age of the preeclamptic and healthy pregnant women to be 29.6 ± 5.7 and 31.9 ± 5.2 years respectively. Similarly, Kristensen et al.[26] observed mean age of case and control groups to be 32 ± 5.0 and 32±4.6 years respectively. In our study majority of subjects in both groups was middle class. Contrary to this finding, Sangeeta et al.[23] mentioned that majority of the women in their study were from the lowest socioeconomic strata (60.0%). Ingec et al.[20] reported that preeclampsia occurs more often in primiparas which compares well with the findings of the present study. In the present study majorities of the cases (85.0%) and controls (90.0%) were preterm (gestational age < 37 weeks) with mean gestational age being 33.2 ± 3.3 and 32.3 ± 3.5 weeks in case and control groups respectively (p > 0.05). However, Franceschini et al.[24] observed higher mean gestational age in their study (35.2 ± 0.4 and 39.5 ± 0.1 weeks in case and control groups respectively). Kristensen et al.[26] showed even higher mean gestational age (39.4 weeks in control and 38.7 weeks in mild preeclamptic group) in their study.

In the present study serum homocystine concentration was significantly raised (15.7 ± 8.3 µmol/L) in case group than that in control group (6.7 ± 1.3 µmol/L) (p < 0.001). However, as none of the control group exhibited abnormally raised serum homocystine (> 15 mmol/L), the risk of having severe preeclmpsia in pregnant women with raised homocystine could not be calculated. Dekker et al.[27] demonstrated that serum homocysteine could be seven times higher in women with severe preeclampsia compared to the normal pregnant women. Similarly, Makedos et al.[28] mentioned that homocysteine concentration was significantly higher in preeclamptic patients than that in the control group (11.11 vs. 6.40 µmol/L, p < 0.001). In another study Powers et al.[29] found a significant difference in the serum levels of homocysteine between preeclamptics and controls (9.0 vs. 7.0 µmol/L, p < 0.05). Furthermore, they found a marker of endothelial dysfunction, with homocysteine. Several other studies have found a positive association of homocysteine with preeclampsia.[30-32] However, these findings were not confirmed by other investigators possibly due to different patient characteristics.[19,20,33] Another systematic review (25 primary articles, 3,649 women), intended to assess the hypothesized mechanism of homocysteine in preeclampsia, showed a positive association of homocysteine serum level with preeclamptic patients in all but one of the review articles, with a weighted mean difference of 2.50 µmol/L (95% CI 1.82– 3.17, P < 0.001) between preclamptic women and healthy pregnant women.[34] In a very recent study[23] the level of serum homocysteine was observed to be higher in the case group compared to control group (10.3 ± 2.5 vs. 3.5 ± 1.2 µmol/L, p = 0.001). The above findings are comparable to those reported by Sanchez et al.[35] and Harma et al.[36] Javadi et al.[22] who showed that the mean serum homocysteine to be 8.9 ± 4.1 µmol/L in patients with severe preeclampsia and 5.5 ± 1.6 µmol/L in control group patients (p < 0.001). Acilmis et al.[37] showed that maternal and fetal serum homocysteine levels to be significantly higher in severe preeclampsia group than those in mild preeclampsia and healthy controls, suggesting that level of serum homocysteine is increased with severity of preeclampsia.

In this study based on the ROC curve, the serum homocysteine levels had the best area under the curve (0.975 or 97.5%) with sensitivity and specificity of the predictor variable being 92.5 and 77.5% respectively at a cut off value > 8 µmol/L. However, Pandya et al.[38] in a ROC curve analysis

showed that the area under the curve (AUC) obtained was 54.0% with sensitivity and specificity of serum homocystine being 61.1 and 49.7% suggesting that the diagnostic accuracy of plasma homocysteine levels in predicting preeclampsia is inappreciably low.

CONCLUSION

The study concluded that homocysteine levels are significantly elevated in patients with preeclampsia compared to the pregnant women without preeclampsia. Homocysteine may be of value in the monitoring of pregnancies to be complicated by pre-eclampsia.

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