



## ASSOCIATION OF SERUM HOMOCYSTEINE LEVELS WITH THE DEVELOPMENT OF PREECLAMPSIA

### Obstetrics and Gynaecology

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### ABSTRACT

**AIMS:** To determine the association between serum homocysteine levels and preeclampsia and its relevance as a potential marker for predicting preeclampsia.

**METHODS:** A total number of 50 preeclampsia cases were taken. 30 cases of normal pregnancy were taken as controls. This study was carried out in the Department of Obstetrics and Gynecology, Patna Medical College and Hospital, Patna from August 2019 to July 2020. Blood samples were drawn from the antecubital vein and serum homocysteine levels were measured.

**RESULTS:** The mean serum homocysteine levels in preeclampsia cases were  $27.7 \pm 6.4 \mu\text{mol/l}$  compared to serum homocysteine levels in normal pregnancy  $9.23 \pm 3.4 \mu\text{mol/l}$  ( $P < 0.01$ ). There was a significant relationship between the systolic and the diastolic blood pressure and serum homocysteine levels. As the systolic and diastolic blood pressure increases, serum homocysteine levels also increased significantly ( $P < 0.001$ ).

**CONCLUSION:** This study showed raised serum homocysteine levels in patients with preeclampsia compared to normal pregnancy.

### KEYWORDS

Serum homocysteine; Preeclampsia; Blood pressure.

### INTRODUCTION

Pre-eclampsia (PE) is a leading cause of maternal and fetal morbidity. Although, the exact cause of pre-eclampsia is still unknown. Recently homocysteine, a metabolite of essential amino acid methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction. Serum homocysteine may prove to be the missing link in the etiology of pre-eclampsia.<sup>1</sup> Decreased antioxidant activity and increased lipid peroxides were shown clearly in pre-eclampsia.<sup>2</sup> Serum concentrations of homocysteine decrease during normotensive pregnancy parallel to the physiologic fall of albumin concentration and folic acid supplementation but increases in pre-eclampsia. In a study conducted on early pregnancy losses, hyperhomocysteinemia was shown to decrease total vessel surface and hence to disrupt placental perfusion.<sup>3</sup> In normal endothelium, nitric oxide (NO) suppresses smooth muscle proliferation in vessel walls. Decreased NO activity by the effect of homocysteine might contribute to the pathology in those patients.<sup>4</sup> It is speculated that a high concentration of homocysteine in preeclampsia is associated with increasing total oxidant and decreasing antioxidant activities which might be the mechanism of endothelial injury and hence vasospasm. Further, homocysteine levels are known to increase with increasing severity of preeclampsia.<sup>5,6</sup> Many studies have supported a positive association between homocysteine and preeclampsia.<sup>7,8</sup> Serum homocysteine concentrations are frequently found to be elevated in cases of preeclampsia but a causal relationship between these two has not been demonstrated yet, hence the study has been taken up with the aim of determining the correlation between serum homocysteine levels and preeclampsia, and its relevance as a potential marker for predicting preeclampsia.

### MATERIALS AND METHODS

This is a case control study carried out in the Department of Obstetrics and Gynecology, Patna Medical College and Hospital, Patna from August 2019 to July 2020. Blood sample was taken from 50 cases of preeclampsia with informed consent. Pregnant women with history of diabetes mellitus, chronic hypertension, renal impairment, liver disease, anemia and other medical illness, history of smoking, and patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants or 6-azauridine triacetate were excluded. Data were collected in a structured proforma for the clinical, laboratory especially serum homocysteine and ultrasonographic variables. Enzyme Cycling Method was used for determining serum homocysteine levels and normal serum homocysteine levels are considered as  $5-15 \mu\text{mol/L}$ .<sup>9</sup> The patient's data was tabulated in a Microsoft excel

sheet and analyzed in STATA. Demographics were noted, means & proportions were calculated. Various statistical tests like Fisher Test, Chi-square test, and Student t-test & correlation was used to find out the meaningful statistical outcomes. Chi-square and Fischer's test were used to significant association between independent variables serum homocysteine and variables like age, the period of gestation, PPBS, Hemoglobin, systolic, and diastolic blood pressure. T-test was used to compare the mean values of serum homocysteine levels. Correlation was used to find relation between causes and serum homocysteine levels.

### RESULTS

The mean serum homocysteine level of 50 pre-eclampsia cases was  $27.7 \pm 6.4 \mu\text{mol/l}$  against  $9.23 \pm 3.4 \mu\text{mol/l}$  ( $p < 0.01$ ) in normal pregnancy. There was a significant relationship between serum homocysteine levels and preeclampsia (mild and severe). Serum homocysteine levels rised with the severity of blood pressure. Multiple causes of preeclampsia were taken into consideration when 3 or more than three factors are present in the same pregnant women. There was a strong association between hyper-homocysteinemia and preeclampsia cases when multiple causes coexist ( $p < 0.001$ ) [Table-1]. Likewise, serum homocysteine levels rised with an increase in the period of gestation in preeclampsia cases ( $p < 0.05$ ).

**Table-1: Serum homocysteine levels between mild and severe preeclampsia (n=50)**

Preeclampsia	Frequency	Mean homocysteine level ( $\mu\text{mol/L}$ )	p-value (unpaired t test)
Mild	32(64%)	25.6	<0.001
Severe	18(36%)	29.9	

On the comparison between the control group with mild PE and severe PE it was found that serum homocysteine levels were elevated in the study group (mild and severe PE) which was statistically highly significant ( $p < 0.001$ ) [Table-2].

**Table-2: Serum homocysteine levels between normal and preeclampsia**

Normal compared with pre-eclampsia	t-value	p-value (unpaired t test)
Mild preeclampsia	10.35	$p < 0.001$
Severe preeclampsia	11.36	$P < 0.001$

As the blood pressure increases either in systolic or diastolic, the serum homocysteine level also raises significantly ( $p < 0.05$ ) [Table3].

**Table-3: Severity of systolic and diastolic blood pressure, and serum homocysteine (n=50)**

	Blood pressure	Frequency	Mean serum homocysteine (μmol/l)	Significance
systolic	150-169	18	24.03	Spearman's rho:0.598,p<0.001, ANOVA test: p<0.001
	170-189	22	28.98	
	190-220	10	32.27	
diastolic	90-99	8	21.03	Spearman's rho:0.517,p<0.001, ANOVA test: p<0.05
	100-109	32	24.83	
	110-120	10	30.48	

The present study has shown a significant positive correlation between systolic (Pearson's rho=0.59, p<0.01) and diastolic (Pearson's rho=0.51, p<0.001) BP and serum homocysteine levels. This suggests that homocysteine levels are directly correlated with the severity of preeclampsia.

## DISCUSSION

From 50 cases studied, the increase in serum homocysteine concentration in pre-eclampsia ( $29.5 \pm 6.3$  vs  $25.6 \pm 5.5$  μmol/l, p<0.001) could be related to the defect in the mechanism that usually decreases homocysteine during normal pregnancy. Our study was supported by the study done by Baksu et al.<sup>10</sup> Sanchez et al.<sup>11</sup> and Powers RW et al.<sup>12</sup> Severity of pre-eclampsia was also significantly correlated with serum homocysteine level that was supported by Hogg et al.<sup>1</sup> Vollset et al.<sup>2</sup> and Lopezquesada et al.<sup>13</sup> but refuted by Hietala et al.<sup>14</sup> Rajmakers et al.<sup>15</sup> and Middeldorp et al.<sup>16</sup> This relationship has been shown in early pregnancy by Cotter et al.<sup>17</sup> in the second trimester by Hogg et al.<sup>1</sup> and Sorensen et al.<sup>18</sup> and in the third trimester by Sanchez et al.<sup>11</sup> and Ingec et al.<sup>19</sup> Concluding that, elevated plasma homocysteine levels in early pregnancy can increase the risk of developing severe preeclampsia by almost threefold. On the other hand, it has been suggested that mid-trimester plasma homocysteine concentrations in asymptomatic women are not predictive in the subsequent development of preeclampsia. Our study was supported by the study conducted by Singh et al.<sup>20</sup> and Rajovic et al.<sup>21</sup> It is possible that in pre-eclampsia, the elevated homocysteine level injures the vascular endothelium which contributes to the pathogenesis of PE. In addition, vascular endothelium in pregnant women may be more sensitive to injury. Therefore, elevation in homocysteine levels may lead to endothelial injury with subsequent activation of various factors that eventually results in pre-eclampsia.<sup>8</sup> In this study, we found a strong association between systolic and diastolic blood pressure and serum homocysteine levels (p<0.05). This was supported by study done by Baksu et al.<sup>10</sup> Further large-scale studies are required, however, to determine whether this modest alteration in homocysteine concentration induced by folic acid supplementation for the whole pregnancy would be effective in reducing adverse pregnancy outcomes. Authors have demonstrated that folic acid supplementation throughout pregnancy suppresses the change in plasma homocysteine concentration which occurred in their study group.<sup>22</sup> This simple, safe, and inexpensive intervention may, therefore, play a preventive role. Further large-scale studies are required to determine the effectiveness of folic acid supplementation in the prevention of poor obstetric outcomes. Elevated levels of homocysteine can be due to genetic or nutritional deficit or a combination of both. Nutritional deficiencies involve inadequate intake of folic acid and vitamin B12.<sup>23</sup> Further studies are required to know the cause of hyperhomocysteinemia (whether nutritional or genetic) observed in pregnant women with pre-eclampsia, abruptio, gestational diabetes mellitus, preterm and IUGR which may help in pharmacological management of pregnant women at risk of fetal loss. Elevated levels of homocysteine can be reduced by administering vitamins which help by increasing the metabolism of homocysteine.<sup>24</sup> The internationally accepted treatment for hyperhomocysteinemia is using a combination of three vitamins-folic acid 400 μg, vitamin B12 500 μg and pyridoxine 10 mg initiating from conception.<sup>25</sup> Further studies are necessary to see whether continuing these agents in the therapeutic dose in the 2nd and 3rd trimester would help to reduce increased levels of homocysteine and keep the associated complications under check.

## CONCLUSION

There was a significant association between serum homocysteine levels and preeclampsia as well as its severity. Serum homocysteine levels were raised in pregnancy with preeclampsia, compared to normal pregnant patients.

## REFERENCES

- Hogg BB, Tamura T, Johnston KE, Debar MB, Goldenberg MA, Goldenberg RL. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. *Am J Obstet Gynecol.* 2000;183:805-9.
- Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, et al. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. *Am J Clin Nutr.* 2000;71:962-8.
- Nelen WL, Bulten J, Steegers EA, Blom HJ, Hanselaar AG, Eskes TK. Maternal homocysteine and chorionic vasculature in recurrent early pregnancy loss. *Hum Reprod.* 2000;15:954-60.
- Kharb S, Gulati N, Singh V, Singh GP. Lipid peroxidation and vitamin E levels in preeclampsia. *Gynecol Obstet Invest.* 1998;46:238-40.
- Wang J, Trudinger BJ, Duarte N, Wilcken DE, Wan XL. Elevated circulating homocysteine levels in placental vascular disease and associated pre-eclampsia. *BJOG.* 2000;107:935-8.
- Kloppenborg RP, Geerlings MI, Visseren FL, Mali WP, Ver-meulen M, Van der Graaf Y, et al. Homocysteine and progression of generalized small vessel disease; the SMART-MR study. *Neurolog.* 2014;82:777-83.
- Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. *Nutr Metab.* 2017;14:78.
- Santilli F, Davi G, Patrono C. Homocysteine, methylenetetrahydrofolate reductase, folate status and atherothrombosis: a mechanistic and clinical perspective. *Vascul Pharmacol.* 2016;78:1-9.
- Varley H, Gowenlock AH, Bell M. *Practical Clinical Bio-chemistry.* Fifth Edition. Jaypee Brothers. India. 1991;1:126-8.
- Baksu A, Taskin M, Goker N, Baksu B, Uluocak A. Plasma homocysteine in late pregnancies complicated with preeclampsia and in newborns. *Am J Perinatol.* 2006;23(1):31-36.
- Sanchez SE, Zhang C, Rene M, Ware-Jauregui S, Lar-rabure G, Williams MA. Plasma folate, vitamin B12 and homocysteine concentrations in preeclamptic and normotensive Peruvian women. *Am J Epidemiol.* 2001;153:474-80.
- Powers R, Evans R, Majors A. Plasma homocysteine concentration is increased in preeclampsia and associated with evidence of endothelial activation. *Am J Obstet Gynecol.* 1998;179:1605-11.
- Lopez Quesada E, Vilaseca MA, Artuch R. Homocysteine and other plasma amino acids in preeclampsia and in pregnancy without complications. *Clin Biochem.* 2003;36(3):185-92.
- Hietala R, Turpeinen U, Laatikainen T. Serum homocysteine at 16 weeks and subsequent preeclampsia. *Obstet Gynecol.* 2001;97:527-9.
- Rajmakers MT, Zusterzeel PL, Steegers EA, Hectors MP, Demacker PN, Peters WH. Hyperhomocysteinemia a risk factor for preeclampsia? *Eur J Obstet Gynaecol Reprod Biol.* 2001;95:226-8.
- Middeldorp S, van de Poel MH, Bank I. Unselected women with elevated levels of factor VIII: C or homocysteine are not at increased risk for obstetric complications. *Thromb Haemost.* 2004;92:787-90.
- Cotter AM, Molloy AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy: a risk factor for the development of severe preeclampsia. *Am J Obstet Gynecol.* 2003;189:391-6.
- Sorensen TK, Malinow MR, Williams MA, King IB, Luthy DA. Elevated second trimester serum homocysteine levels and subsequent risk of preeclampsia. *Gynecol Obstet Invest.* 1999;48:98-103.
- Ingec M, Borekci B, Kadanali S. Elevated plasma homocysteine concentrations in severe preeclampsia and eclampsia. *Tohoku J Exp Med.* 2005;206(3):225-31.
- Singh U, Gupta HP, Singh RK. Homocysteine Association with preeclampsia and normotensive pregnancy. *J Obstet Gynecol.* 2009;59(3):235-8.
- Rajkovic A, Mahomed K, Malinow MR, Sorensen TK, Woelk GB, Williams MA. Plasma homocysteine concentrations in eclamptic and preeclamptic African women postpartum. *Ob-stet Gynecol.* 1999;94:355-60.
- Ambrosino P, Lupoli R, Di Minno A, Nardo A, Marrone E, Lupoli V, et al. Cyclic supplementation of 5-MTHF is effective for the correction of hyperhomocysteinemia. *Nutr Res.* 2015;35:489-95.
- Sirdah MM, Yassin MM, Shekhi SE, Lubbad AM. Homocysteine and vitamin B12 status and iron deficiency anemia in female university students from Gaza Strip, Palestine. *Rev Bras Hematol Hemoter.* 2014;36(3):208-12.
- McCaddon A. Homocysteine and cognitive impairment; a case series in a General Practice setting. *Nutr J.* 2006;5:1-6.
- Micle O, Muresan M, Antal L, Bodog F, Bodog A. The influence of homocysteine and oxidative stress on pregnancy outcome. *Journal of Medicine and Life.* 2012;5(1): 68-73.