



## STUDY OF SERUM HOMOCYSTEINE, FOLIC ACID AND VITAMIN B12 IN PATIENTS OF PREECLAMPSIA

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### Abstract

**Background:** Preeclampsia is a pregnancy specific syndrome involving vasospasm and endothelial activation. It is a global problem affecting at least 10% of all the pregnancies. It is a multisystem progressive disorder and is among the leading cause of maternal and perinatal morbidity and mortality. Hypertension and proteinuria are the most prominent features of Preeclampsia. Elevated levels of Homocysteine during gestation have been linked to adverse outcomes in pregnancy like Preeclampsia. Folate and vitamin B12 status has also been related to the occurrence of neural tube defects.

**Aim:** The present study was planned to evaluate the levels of Serum Homocysteine, Folic acid and Vitamin B12 levels and their association in patients with preeclampsia.

**Methodology:** 50 pregnant females diagnosed with preeclampsia were enrolled for the study based on predefined inclusion and exclusion criteria. Age matched Normotensive pregnant women (n=50) constituted the control group. Blood Samples were collected and analyzed for serum Homocysteine, Folic acid and Vitamin B12. Results obtained were analyzed by applying suitable statistical tests.

**Result:** Serum Homocysteine levels were significantly higher in the preeclamptic group as compared to control group ( $p < 0.0001$ ) whereas Serum Folic acid and Vitamin B12 levels were significantly lower. A highly significant correlation ( $r = -0.469$ ;  $P < 0.0001$ ) was observed between serum Homocysteine and Vitamin B 12 among the preeclamptic group.

**Conclusion:** The study suggests a statistically significant increase in Homocysteine and decreased in Folic acid and Vitamin B12 levels. Monitoring of these biochemical parameters during follow up of Preeclampsia treatment is recommended for proper clinical management. Moreover, screening of these markers at an early stage of pregnancy can predict the risk of developing preeclampsia which may help in timely management and aversion of associated complications.

**Keywords:** Pregnancy, hypertension, microalbuminuria, eclampsia, intra uterine growth retardation, hyperhomocysteinemia

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DOI: 10.48047/ecb/2023.12.si5a.0613

## INTRODUCTION

Pregnancy is the period from conception to birth which usually lasts up to 40 weeks. After the egg is fertilized by a sperm and then implanted in the lining of uterus it develops into the placenta and embryo and later into the foetus. Pregnancy is divided into three trimesters and each lasts for three months. 10-15% of all pregnancies are complicated due to hypertension. Among them, 10-20 % develop proteinuria. Pregnancy induced Hypertension, also known as preeclampsia (PE) is a complication of pregnancy which has an adverse effect on both, mother and foetus.

Preeclampsia is defined as blood pressure equal to or higher than 140/90 mmHg with proteinuria of either higher than 100 mg/dl by urine analysis or higher than 300 mg in a 24-h urine collection. Severe preeclampsia is defined as blood pressure equal to or higher than 160/110 mmHg. It is a condition that develops in previously normotensive pregnant women after 20 weeks of gestation, and is characterized by onset of hypertension and proteinuria. If left untreated, preeclampsia can progress to a convulsive state known as eclampsia. Although, the exact cause of preeclampsia is not known, the basic pathology is endothelial dysfunction and intense vasospasm. [1]

Homocysteine, a sulphur containing amino acid, is an immediate product of methionine metabolism. High maternal homocysteine level (Hyperhomocysteinemia) causes endothelial damage and dysfunction, platelet dysfunction, thrombus formation and smooth muscle proliferation. Probably, this causes increased incidence of Preeclampsia, miscarriage, Intra uterine growth restriction, placenta abruption, low birth weight, etc. [2] Folic acid and Vitamin B12 have a key role in methionine-homocysteine metabolism. Folic acid fortification in the diet of pregnant woman is advisable since the first trimester. Deficiency of folate may increase the risk of complications of pregnancy as well as development of foetus. Similarly, there is increasing evidence that inadequate or deficient vitamin B12 status might be associated with complications of pregnancy [3].

The present study was planned to evaluate the role of Homocysteine, Folic acid Vitamin B12 in patients with Preeclampsia.

## MATERIALS & METHODS

The study was conducted on fifty preeclamptic pregnant females diagnosed on the basis of hypertension and/ or proteinuria. All selected females were normotensive before conception. Fifty age matched, normotensive pregnant women constituted the control group. The study was conducted after seeking approval from institutional ethics committee and informed written consent of all subjects enrolled. Patients with chronic hypertension, diabetes mellitus, severe anemia, multifocal pregnancy, abruptio placenta, preterm labor and delivery, smoking, liver disease & not willing to participate were excluded from the study.

Blood Samples were collected through venipuncture using standard aseptic techniques and analyzed for serum Homocysteine, Folic acid and Vitamin B12. All investigations were conducted on VITROS 5600 analyzer. Results obtained were presented as mean  $\pm$  SD and compared among case and control group by applying student's t-test. Association among study parameters was evaluated by applying Pearson's correlation. All statistical evaluations were performed using IBM SPSS Statistics version 23

## RESULT

Various parameters were compared between the case and control group by applying student's t-test (Table 1). The mean age between case and control group were comparable (P=NS). Systolic and Diastolic blood pressures were observed to be significantly higher in preeclamptic women as compared to normotensive pregnant females. Serum Homocysteine levels were compared between cases and control and observed to be significantly increased in cases.

Serum Folic acid and Vitamin B12 levels were observed to be significantly lower in the preeclamptic group as compared to control group.

To evaluate the correlation of serum Homocysteine levels with vitamin B12 & Folic acid levels, Pearson's correlation was applied (Table 2). A statically significant negative association ( $r = -0.469$ ) was observed between Homocysteine and Vitamin B12. However non-significant correlation was found between Homocysteine and Folic acid ( $r = 0.176$ ; P=NS).

**Table 1: Comparison of various parameters in Preeclampsia Cases and Control group**

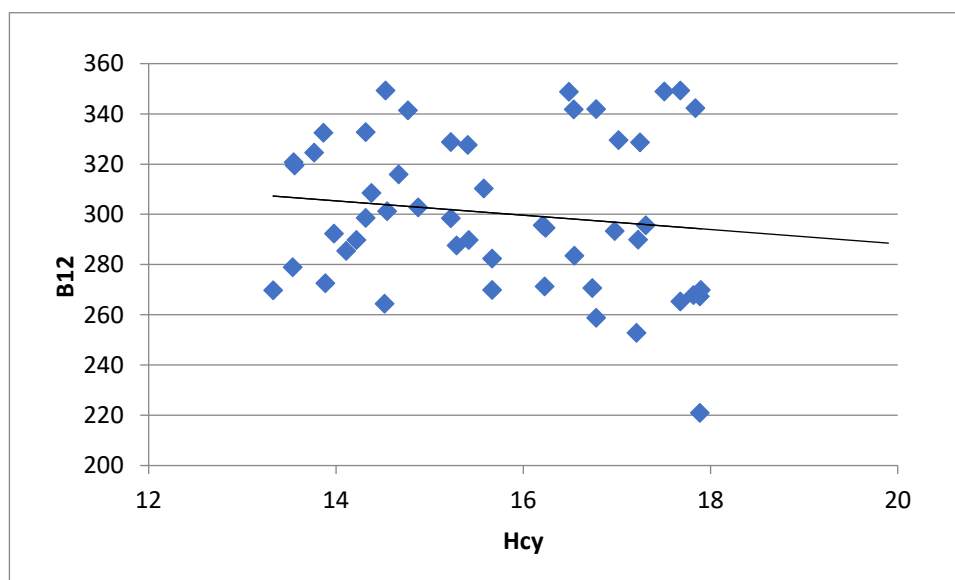
Parameters	Cases (n=50)	Control (n=50)	P value
Age (years)	27.10±3.56	27.64±3.34	NS
Systolic BP (mmHg)	149.62±7.80	128.88±5.60	<0.0001
Diastolic BP (mmHg)	94.20±5.20	75.18±6.06	<0.0001
Homocysteine (μmol/L)	15.72±1.46	7.90 ± 0.83	<0.0001
Folic acid (ng/ml)	6.88±1.46	13.75 ± 1.79	<0.0001
Vitamin B12 (pg/dl)	300.46±30.53	659.51±23.45	<0.0001

P- value as obtained on applying Student's t test

**Table 2: Correlation of serum Homocysteine levels with Folic acid & Vitamin B12**

	Correlation coefficient (r)	P-value
Hcys vs Folic acid	0.176	NS
Hcys vs Vit B12	-0.469	<0.0001

P- value as obtained on applying Pearson's Correlation

**Figure 1: Correlation of Hcys and Vitamin B12**

## DISCUSSION

Blood pressure in pregnancy has different patterns.

In normal pregnancies, there is a gradual, small decrease in blood pressure during the first 30 weeks and then an increase with levels of systolic and diastolic blood pressure slightly above the initial values at the end of pregnancy. [4] When it reaches a level of 140/90 mmHg for systolic/diastolic pressure and is accompanied by proteinuria, the condition is known as Preeclampsia (PE). Severe Preeclampsia is defined as systolic blood pressure  $\geq 160$  mmHg and diastolic blood pressure  $\geq 110$  mmHg. [5]

As expected, mean systolic and diastolic blood pressure of preeclamptic group was significantly higher as compared to normotensive control group. Similar pattern

in systolic and diastolic BP were reported by **Maria Bullarbo et al (2014)**. [6] **J. Gunnarsdottir et al (2019)** concluded in their study that elevated diastolic BP from early to mid-gestation was associated with increased risks of preeclampsia and small for gestational age (SGA), especially for women also delivering preterm. The results may imply that the diastolic BP starts to increase around mid-gestation in women later developing placental dysfunction disorders. [7]

Homocysteine is sulphur containing nonproteinogenic  $\alpha$ -amino acid required for the growth of cells and tissues in the body. It is homologue of cysteine but differs by an added methylene bridge and formed during the metabolism of methionine to cysteine. [8]

Homocysteine is critically important during pregnancy. Normally maternal levels of serum Homocysteine decreases as gestational age increases. It may be due to physiological pregnancy response or maybe due to decreased albumin, increased estrogen, hemodilution occurring as a result of increase in plasma volume and increased methionine demand by mother and foetus.

The present study observed significantly higher homocysteine levels among preeclamptic females. Homocysteine promotes the production of reactive oxygen species, also known as free radicals, which results in oxidative stress and damage of vascular endothelial cells, thus causing systemic arterial spasm and other pathophysiological manifestations, which conforms to the pathogenesis of preeclampsia. An anticoagulant property of the endothelium blunts the response to the agonist by releasing nitric oxide (NO<sub>2</sub>). Endothelial dysfunction causes platelet dysfunction, thrombus formation and smooth muscle proliferation. The incidence of hyperhomocysteinemia in Indian population is about 54%. The prevalence of preeclampsia and hyperhomocysteinemia is high among Indian population [9]. Results similar to the present study were reported by **Md Mozammel Hoque et al** (2008) [10], **Gurbuz et al**, (2004) [11] and **Pi-Sunyer FX et al** (2000) [12]. Another study by **Lokeshwari Kannayan, et al** (2020) demonstrated the significant association between serum homocysteine levels and preeclampsia as well as its severity [13].

**S. Naga Jyothi, et al** (2018) stated in their study that maternal serum homocysteine levels were significantly increased in Preeclampsia compared to normotensive subjects. Serum homocysteine was significantly raised in severe preeclampsia than in mild preeclampsia and shows association between serum homocysteine and severity of Preeclampsia. [14] **Shilpa A.V et al** (2017) concluded in their study that levels of serum homocysteine in pre eclampsia were lower compared to non-pregnant normal woman. They concluded a positive association between homocysteine levels in preeclamptic women and normotensive women and infer the role of oxidative stress in its pathogenesis. [15]

### Folic acid in Preeclampsia

Folic acid is said to be critically important for foetal development. It acts as a cofactor for many essential cellular reactions including the transfer of single carbon units; it is required for cell division because of its role in DNA synthesis. Folate is also a substrate for a variety of reactions that affect the metabolism of several amino acids, including the transmethylation and transsulfuration pathways. A central feature of foetal development is widespread and sustained cell division. As a result of its role in nucleic acid synthesis, the need for folate increases during times of rapid tissue growth. During pregnancy, folate-dependent processes include an increase in red cell mass, enlargement of the uterus, and growth of the placenta and foetus [16]. Folic acid deficiency may induce the apoptosis of human cytotrophoblast cells, thus possibly affecting trophoblastic invasion and placental development. Therefore, the supplementation of folic acid may improve placental implantation and subsequently affect the incidence of hypertensive pregnancy disorders. Supplementation of folic acid has been found to decrease preeclampsia risk. The possibility is that folic acid can affect the levels of hyperhomocysteinemia, which is suggested to damage the vascular endothelium of the developing placenta. [17]

Serum folate levels were reportedly lower in the preeclamptic women group as compared to the normotensive pregnant women. Similar results were reported by **Nahid Shahbazian et al** (2016). [18] Another study by **Cheng Liu et al** (2018) indicated that the supplementation of multivitamins containing folic acid during pregnancy could significantly lower preeclampsia risk. [19] **Norma C.et al** (2018) concluded in their study that maternal serum concentrations of folate were associated as a protective factor for preeclampsia while concentrations of homocysteine were associated as a risk factor. [20] Another study by **Shi Wu Wen, Yanfang Guo et al** (2016) reported that folic acid supplementation in pregnancy was associated with reduced risk of preeclampsia and the association was statistically significant only in women with increased risk of developing preeclampsia. [21] **Sang-Min Shim et al** (2016) demonstrated in their study that multivitamin containing folic acid or folic acid alone was not significantly effective in

reducing gestational hypertension or Preeclampsia incidence. [22]

### Vitamin B12 in Preeclampsia

Vitamin B-12, also known as cobalamine, is a micronutrient essential for cellular growth, differentiation and development. It has crucial for normal cell division and differentiation, and necessary for the development and myelination of the central nervous system. [23] Poor vitamin

B12 status in infancy is linked to poor growth and neurodevelopment and increased homocysteine concentration which will lead to preeclampsia. Brain development starts from conception, and pregnancy is a period of rapid growth and development for the brain. Vitamin B12 and folate act as methyl donors in one-carbon metabolism which affects cell growth and differentiation by influencing DNA synthesis and epigenetic regulation. [24]

Similar results were reported by **Nahid Shahbazian et al (2016)**. [18] A study by **Hongyan Zhao, Xu Zeng (2019)** concluded that folic acid and vitamin B12 may play an important role in the development of Preeclampsia and could be potential serological biomarker for early preeclampsia diagnosis. [25] Another study by **Laivouri et al. 1999** represented a decreased in serum B12 levels in Preeclamptic patients. [26]

Similar study done by **Shahid A. et al 2011** [27] they observed in their study that the serum homocysteine was found to have negative and insignificant correlation with serum folic acid in preeclamptic patients. Their correlation coefficient (r) of -0.196 (P = 0.174). A good, negative and statistically significant correlation (r = -0.335 and P=0.05) was found between serum homocysteine and vitamin B12 in preeclampsia. Another study done by **Nahid Shahbazian (2016)** [18] in this study, a significant inverse correlation was found between homocysteine and serum folate. In addition, a negative non-significant correlation occurred between levels of vitamin B12 and homocysteine.

### CONCLUSION

The study suggests that high serum Hcys and decreased Vitamin B12 and folate have a strong association with the state of preeclampsia. It is, therefore, recommended

that these parameters should be evaluated in the initial weeks of gestation so that expecting mothers with risk of developing preeclampsia can be identified at an early stage of pregnancy. Early identification of patients who are at risk of developing preeclampsia and later eclampsia can be given proper medical advice such that the associated complications may be averted.

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