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Original Research Article

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

Parveen S Kavathekar[1], Ranjana C Shingne[2], Santosh Kumar Jha[3], Apurva Sakarde[4]

[1]Assistant Professor, Department of Biochemistry, Government Medical College, Aurangabad, Maharashtra, India

[2]Professor, Department of Physiology, Dr Ulhas Patil Medical College and Hospital, Jalgaon, Maharashtra, India

[3]Assistant Professor, Department of Physiology, Dr Ulhas Patil Medical College and Hospital, Jalgaon, Maharashtra, India.

[4]Assistant Professor, Department of Biochemistry, All India Institute of Medical Sciences, Nagpur, Maharashtra, India.

Corresponding Author: Ranjana C Shingne
Email id: Ushanka08@gmail.com

Abstract

Introduction: Preeclampsia is a major cause of maternal or fetal morbidity and mortality. Its pathophysiology is still not clear. Homocysteine is a sulfur containing amino acid, which damages smooth muscle of the vessel wall. The objective of this study was to correlate serum homocysteine with vitamin B12 and folic acid level in preeclampsia and normal pregnant women.

Materials and Methods: The study reviewed a total of 150 women with pregnancy. These participants are categorized into three groups: healthy pregnant group, mild preeclampsia group and severe preeclampsia group. The three study groups were statistically similar in aspects of maternal and gestational age.

Results: The mean serum homocysteine level was found 15.67 ± 0.73 µmol/l in mild preeclampsia group 19.18 ± 0.46µmol/l in severe preeclampsia group and 12.44 ± 0.39 µmol/l in the group of normal pregnant women.

Conclusion: Serum homocysteine levels were significantly increased in patients with mild and severe preeclampsia than the healthy normotensive pregnant women. Serum homocysteine showed significant positive correlation with systolic and diastolic blood pressure in mild and severe preeclampsia.

Key words: Homocysteine, vitamin B12, folic acid, preeclampsia

Introduction

Preeclampsia is a hypertensive disease that occurs during pregnancy. This disease accounts of greater than 50,000 maternal deaths, and over 5,00,000 fetal deaths worldwide. The worldwide incidence of preeclampsia is 5-8% of all pregnancies[1]. In India, the incidence of preeclampsia is reported to be 8-10% of all pregnancies[2]. Preeclampsia is defined as new onset hypertension. The parameters of initial identification of pre-eclampsia are specifically defined as a systolic blood pressure of 140 mm Hg or more or diastolic blood pressure of 90 mm Hg or more on two

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occasions at least 4 hours apart; or shorter interval timing of systolic blood pressure of 160 mm Hg or more or diastolic blood pressure of 110 mm Hg, all of which must be identified after 20 weeks of gestation.

The initial presentation of preeclampsia typically arises in near term pregnancies. Other significant findings that may or may not be a part of the clinical presentation include proteinuria, signs of end organ damage such as thrombocytopenia, impaired liver function and severe persistent right upper quadrant or epigastric pain . Delayed delivery of the fetus in preeclamptic patients in the late pre-term period increases the risk of severe hypertension, with severe consequences such as eclampsia, HELLP syndrome, pulmonary edema, myocardial infarction, acute respiratory distress syndrome, stroke, renal and retinal injury, and fetal complications including fetal growth restrictions, placental abruption, or fetal or maternal death[3,4].

Homocysteine, a sulfur containing amino acid, is involved in processes such as lipid peroxidation and oxidative stress[3]. It has been hypothesized that maternal hyperhomocysteinemia is associated with a number of placenta mediated diseases such as pre-eclampsia[5,6]. An increased concentration of total circulating homocysteine in serum is recognized as an independent risk factor for cardiovascular disease[7]. Hyperhomocysteinemia is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis and occlusive vascular disease[8] .The vascular changes induced by homocysteine are similar to those associated with preeclampsia. It includes atherosis and endothelial dysfunction resulting in blunted vasorelaxation mechanisms[9].

Homocysteine concentrations are tightly regulated by 2 enzymatic pathways. Homocysteine can be remethylated to methionine by a pathway requiring folic acid as a methyl donor. In addition to folic acid the pathway requires vitamin B12 as an important cofactor. Alternatively homocysteine can be removed by trans-sulfuration, a pathway dependent on cofactor vitamin B6. Enzymatic defects in either of these pathway results in increased homocysteine levels. Deficiency of folic acid, vitamin B12 or B6 also leads to increase in homocysteine levels[10]. Vitamin B12 and folate play a vital role in one carbon (1-C) metabolism which is crucial for the general wellbeing of pregnancy and particularly for fetal growth[11]. Moreover, determinants of hyperhomocysteinemia such as low concentration of vitamin B12 and folic acid involved in homocysteine metabolism are also associated with increased risk of vascular damage[7].

So biochemical parameters include in this study are serum homocysteine, vitamin B12 and folic acid. Studying the differences in biochemical parameters serum homocysteine, vitamin B12 and folic acid between normal pregnancies and those complicated by preeclampsia may give important information that can be useful for finding a possible predictor of preeclampsia. Identification of such a predictor would help to intervene and reduce the consequences of this disorder. The aim of our study is to determine the serum homocysteine levels in preeclampsia and its correlation with vitamin B12 and folic acid levels.

MATERIALS AND METHODS

The present case control study was conducted in the Department of Biochemistry, Government Medical College & Hospital during the period of January 2014 to October 2015. Permission of the institutional ethical committee was taken prior to the study. After written and informed consent, total 150 subjects were included in this study. The study population was divided into 3 groups: Group I- 50 Healthy normotensive pregnant women as control, Group II- 50

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Diagnosed cases of mild pre-eclampsia and Group III - 50 Diagnosed cases of severe preeclampsia.

Group I (Controls): Consisted of 50 normotensive and non-proteinuric healthy pregnant women attending the outpatient dept. of obstetrics, Govt. Medical College for regular antinetal checkup. Subjects developing hypertension at any time during the antinetal follow up were excluded from the study.

GROUP II (cases of mild preeclampsia): Consisted of pre-eclamptic women having a systolic BP of ≥140 but <160 mm Hg and a diastolic BP of ≥ 90 but <110 mm Hg and 24 hours urinary protein excretion of ≥ 0.3 gms corresponding to 1+ dipstick or greater.

GROUP III (cases of severe preeclampsia): Consisted of pre-eclamptic women having a systolic BP of >160 mm Hg and a diastolic BP of >110 mm Hg on two occasions six or more hours apart and 24 hours urinary protein excretion of ≥ 5 gms or 3+ dipstick or greater on two random samples collected four or more hours apart. Other signs and symptoms of multiorgan involvement such as headache, visual disturbances, epigastric pain, etc. may be present.

Inclusion criteria

1. Already diagnosed cases of preeclampsia
2. Age group between 18 to 35 years
3. Single gestation with gestational age > 20 weeks
4. Patient taking folate supplement and mixed diet

Exclusion criteria

1. Pre-eclampsia in previous pregnancy
2. History of pre-term labour, abruption and miscarriage in previous pregnancy
3. History of anaemia, haemolytic disease and any thyroid disorder
4. Multiple (twins, triplet etc.) pregnancy and molar pregnancy

Biochemical investigations -After obtaining a written informed consent and taking all aseptic precautions, fasting venous blood samples were collected from all participants in the plain bulb. Serum from plain bulbs was separated after one hour by centrifugation at 3000 rpm for 10 minutes and was tested for serum homocysteine, serum vitamin B 12 and serum folic acid. Two levels of quality controls were run for all the parameters. Instrument used for serum homocysteine and vitamin B12 analysis is ADVIA Centaur CP by Siemens fully automated immunoassay system. Folic acid analysis was done by Monobid semi automated immunoassay system.

RESULTS

Demographic and biochemical characteristics of all the participants were analyzed as mean ± standard deviation. Unpaired t test was applied to analyze the differences of studied characters in study groups. P value was obtained from the unpaired t test as > 0.05 not significant, < 0.05 significant and < 0.01 highly significant.

Correlation coefficients (r) were calculated among various parameters in preeclamptic and control groups. Positive and negative r values were interpreted as follows: r = 0 (no correlation), r = 0 to 0.3 (poor correlation), r = 0.3 to 0.7 (considerable correlation) and r = 0.8 or more (strong correlation).

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Table 1 shows that mean values of maternal age (P=0.15) and gestational age (P=0.19) did not differ significantly among the studied groups. Whereas mean values of systolic BP and diastolic BP (P< 0.0001) are significantly increased in group II than controls (group I). Urinary protein is nil in group I and 1+ in group II.

Table 1: Comparison of demographic parameters in studied groups

Parameter	Group I- Control	Group II -Mild Preeclampsia	P value
	MEAN ± SD		
Maternal age (yrs)	23.60 ± 0.43	22.74 ± 0.40	0.15
Gestational age (weeks)	33.34 ± 0.33	33.78 ± 0.27	0.19
Systolic BP (mm of Hg)	117.9 ± 1.17	147.1 ± 0.66	<0.0001**
Diastolic BP (mm of Hg)	74.04 ±0.98	97.64 ± .57	<0.0001**
Urine protein	Nil	1+	

*Significant P value
** Highly significant P value

Table 2: Comparison of demographic parameters in studied groups

Parameter	Group II-Mild Preeclampsia	Group III -Severe Preeclampsia	P value
	MEAN ± SD		
Maternal age (yrs)	22.74 ± 0.40	22.44 ± 0.42	0.61
Gestational age (weeks)	32.78 ± 0.27	32.52 ± 0.26	0.50
Systolic BP (mm of Hg)	147.1 ± 0.66	168.2 ± 0.76	<0.0001**
Diastolic BP (mm of Hg)	97.64 ± 0.57	118.8 ± 0.70	<0.0001**
Urine protein	1+	3+	

*Significant P value
** Highly significant P value

Table 2 shows that mean values of maternal age (P=0.61) and gestational age (P=0.50) did not differ significantly among the studied groups. Whereas mean values of systolic BP and diastolic BP (P< 0.0001) are significantly increased in group III (severe PE) than group II (mild PE). Urinary protein is 1+ in group II and increases to 3+ in group III.

Table 3: Comparison of demographic parameters in studied groups

Parameter	Group I- Control	Group III -Severe Preeclampsia	P value
	MEAN ± SD		
Maternal age (yrs)	23.60 ± 0.43	22.44 ± 0.42	0.06
Gestational age (weeks)	33.34 ± 0.32	32.52 ± 0.26	0.05
Systolic BP (mm of Hg)	117.9 ± 1.17	168.2 ± 0.76	<0.0001**
Diastolic BP (mm of Hg)	74.04 ±0.98	118.8 ± 0.70	<0.0001**
Urine protein	Nil	3+	

*Significant P value
** Highly significant P value

Table 3 shows that mean values of maternal age (p=0.06) and gestational age (p=0.05) did not

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differ significantly among the studied groups. Whereas mean values of systolic BP and diastolic BP (P< 0.0001) are significantly increased in group III than controls (group I).Urinary protein is nil in group I and increases to 3+ in group III.

Table 4: Comparison of biochemical parameters in studied groups.

Parameter	Group I - Control	Group II - Mild Preeclampsia	P value
	MEAN ± SD		
Homocysteine(µmol/l)	12.44 ± 0.39	15.67 ± 0.73	0.0002**
Folic acid (ng/ml)	10.99 ± 0.28	9.93 ± 0.24	0.005
Vitamin B12(pg/ml)	380.1 ± 5.36	349.1 ± 5.84	0.0002**

*Significant P value
** Highly significant P value

Serum homocysteine is significantly increased (p=0.0002) in mild PE (group II) than in controls (group I). Folic acid and vitamin B12 levels are significantly decreased in mild PE (group II) than in controls (P=0.005 and P<0.0002 respectively).

Table 5: Comparison of biochemical parameters in studied groups

Parameter	Group II- Mild Preeclampsia	Group III- Severe preeclampsia	P value
	MEAN ± SD		
Homocysteine(µmol/l)	15.67 ± 0.73	19.18 ± 0.46	0.0001**
Folic acid (ng/ml)	9.93 ± 0.24	9.23 ± 0.23	0.04*
Vitamin B12(pg/ml)	349.1 ± 5.84	316.8 ±4.18	<0.0001**

*Significant P value
** Highly significant P value

Serum homocysteine is significantly increased (p=0.0001) in severe PE (group III) patients than in mild PE (group II). Folic acid and Vitamin B12 levels are significantly decreased in severe PE (group III) than in mild PE (group II) (p=0.04, p=0.0001 respectively).

Table 6: Comparison of biochemical parameters in studied groups

Parameter	Group I -Control	Group III- Severe Preeclampsia	P value
	MEAN ± SD		
Homocysteine (umol/l)	12.44 ± 0.39	19.18 ± 0.46	<0.0001**
Folic acid (ng/ml)	10.99 ± 0.28	9.23 ± 0.23	<0.0001
Vitamin B12 (pg/ml)	380.1 ± 5.36	316.8 ±4.18	<0.0001**

*Significant P value
** Highly significant P value

Serum homocysteine is significantly increased (p<0.0001) in severe PE (group III) than in controls (group I). Folic acid and Vitamin B12 levels are significantly decreased in severe PE (group III) than in controls (p<0.0001).

Table 7: Correlation of various parameters in group II (Mild PE)

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Parameters	r value	P value
Homocysteine vs Systolic BP	0.52	0.0001**
Homocysteine vs Diastolic BP	0.50	0.0002**
Homocysteine vs Folic acid	-0.35	0.01*
Homocysteine vs Vitamin B12	-0.495	0.0003**

Serum homocysteine is positively correlated with both systolic and diastolic BP (r=0.52, p= 0.0001 and r=0.50, p=0.0002 respectively) while it is negatively correlated with folic acid (r= -0.35, p=0.01) and vitamin B12 (r=- 0.495, p=0.0003).

Table 8: Correlation of various parameters in group III (severe PE)

Parameters	r value	P value
Homocysteine vs Systolic BP	0.53	<0.0001**
Homocysteine vs Diastolic BP	0.53	<0.0001**
Homocysteine vs Folic acid	-0.356	0.01*
Homocysteine vs Vitamin B12	-0.600	<0.0001**

Serum homocysteine is positively correlated with both systolic and diastolic BP(r=0.53, p=<0.0001 and r=0.53, p=<0.0001 respectively) while it is negatively correlated with folic acid (r=-0.356, p=0.01) and vitamin B12(r=- 0.600, p=<0.0001).

DISCUSSION

Pre-eclampsia is a multisystem disorder of unknown etiology characterized by the onset of hypertension ≥140/90 mmHg with proteinuria after the 20th week in a previously normotensive and non-proteinuric patient. The pathophysiology of pre-eclampsia is poorly understood. But currently endothelial dysfunction is most popularly hypothesized to be a central pathophysiological feature of pre-eclampsia. It leads to altered vascular reactivity, loss of vascular integrity and activation of the coagulation cascade[5].

Homocysteine , a thiol-containing amino acid, is the demethylated derivative of the essential amino acid methionine and thus an intermediate in the methionine cycle. Elevated homocysteine is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis and occlusive vascular disorders[5]. Hyperhomocysteinaemia has been regarded as a new modifiable risk factor for vascular disease[12].

This study was aimed to identify the role of homocysteine as a predictor of preeclampsia, which can be used for screening purpose so that high risk women can be identified at an early stage to prevent their progression to complications. We also aimed at correlating total homocysteine level with vitamin B12 and folic acid and to evaluate its association with preeclampsia.

In our study, serum homocysteine levels were significantly increased in both groups of preeclampsia (group II and group III) than controls (p<0.001). Serum homocysteine levels was significantly increased in severe preeclampsia than mild (p<0.001). Homocysteine is a sulphur containing amino acid. It is derived from demethylation of methionine which an essential amino acid. It requires folate, vitamin B12 and B6 as co-enzymes. Malnutrition or malabsorption of folate and/or vitamin B12 or B6 or inherited enzymatic defects within the methionine-homocysteine pathway such as methylene tetrahydrofolate reductase or cystathionine β- synthase deficiency leads to raised homocysteine levels[13].

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In the current study, mean serum homocysteine was 12.44 ± 0.39 for control group, 15.67 ± 0.73 for mild preeclampsia and 19.18 ± 0.46 in severe preeclampsia group. A study conducted by Badrunnahar Rumi et al found the mean serum homocysteine levels in normal pregnancy, mild preeclampsia and severe preeclampsia are 8.2±3.7µmol/l, 16.4±7.9µmol/l and 20.8±8.0µmol/l respectively[14]. 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 respectively and 12.48±2.95 µmol/l in control group[15]. Kabra et al, (2015) in their study found serum homocysteine levels showed a highly significant increase with the severity of pre-eclampsia. Mean serum Homocysteine level for the mild pre eclamptic group was 13.20 +3.68 µmol/L and for the severe pre-eclampsia group was as high as 23.95 + 9.85 µmol/L[16]. In a similar study by Khosrowbeygi A et al, (2011) found that women with severe pre-eclampsia had higher serum levels of total homocysteine than mild preeclamptic patients (17.40±2.7 vs 11.49±1.19 µmol/l) and normal pregnant women (17.40±2.7 vs 6.38±0.3 µmol/l)[5]. In the present study there is significant positive correlation (r=0.52, p= 0.0001) was found between serum homocysteine and systolic blood pressure.

Other similar studies showed a positive correlation between serum homocysteine and systolic blood pressure[14,17,18] . Present study has significant positive correlation (r=0.50, p=0.0002) between diastolic blood pressure and serum homocysteine level, which is similar with some other studies [18,19].

In our study, vitamin B12 levels were significantly decreased in both groups of preeclampsia (group I and group II) than control group. Vitamin B12 deficiency is frequently reported in pregnancy due to inadequate dietary intake of vitamin B12. In third trimester of pregnancy there is a gradual decline in the serum concentration of vitamin B12 due to hemodilution, hormonal changes, increased maternal metabolic rate, alterations in the concentration of vitamin B12 binding proteins, and placental transport of vitamin B12 to the fetus[20]. Arpita P Patel et al (2012), in their study found that mean levels of vitamin B-12 decreased in preeclampsia group (157.9± 58.13) than the control group (243.7 ± 60.90)[21]. Similar study conducted by Mujawar SA and colleagues found that mean level of vitamin B12 significantly decreased when control group (391 ± 105) was compared with study group (321±64.9) of preeclampsia [7].

Folic acid level was significantly decreased in both groups of preeclampsia (group I and group II) than control group. Folic acid level was significantly decreased in severe preeclampsia than mild preeclampsia. Folate deficiency can occur because of low dietary intake or increased metabolic requirement by a particular genetic defect or defects[22]. Incorrect and irregular use of folic acid supplements may lead to folate deficiency[23]. Malnutrition or malabsorption may be the another cause of folate deficiency[24]. Folic acid can also prevent and reverse endothelial dysfunction, independent of plasma homocysteine.

This suggests that folic acid supplements may have a role in the prevention of preeclampsia [25]. Salehi-Pourmehr H et al (2012) in their study observed the serum levels of folic acid in preeclampatic and the non- preeclampatic women. The mean of folic acid serum levels in preeclamptic group (10.9 ± 3.9) cases was significantly lower than non-preeclamptic group (13.6 ± 4.0)[23]. In a similar study conducted by Arpita P Patel and colleagues found that mean level of folic acid is decreased in preeclampsia group (9.5 ± 4.42) as compared to the control group (6.4±2.54)[21].

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CONCLUSION

In the present study we observed that serum homocysteine levels were significantly increased in patients with preeclampsia (both mild and severe) than the healthy normotensive pregnant women. Vitamin B12 and folic acid levels were significantly decreased in patients with mild and severe preeclampsia than the normal healthy pregnant women. Systolic and diastolic blood pressures were significantly increased in patients with preeclampsia (mild and severe) than healthy pregnant women. Serum homocysteine showed significant positive correlation with systolic and diastolic blood pressure in mild and severe preeclampsia. Hyperhomocystenemia, reflection of endothelial damage dysfunction and vascular damage plays important role in pathophysiology of preeclampsia. Our results also emphasize high cardiovascular risk in patients with preeclampsia, which is supported by increase in serum homocysteine level in these patients. Elevated level of serum homocysteine can be due to nutritional defect. Nutritional defects involve inadequate intake of vitamin B12 and folic acid. So, deficiency of vitamin B12 and folic acid might have a role in pathogenesis of preeclampsia.

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