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Original Research Article

A comparative study of serum homocysteine levels in normal versus severe preeclampsia at term gestation

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Abstract
Hyperhomocysteinemia has been known to cause many vascular diseases. But the exact pathogenesis of Hyperhomocysteinemia induced vascular damage is not clearly known. Several factors such as oxidative stress, endothelial dysfunction, smooth muscle cell proliferation and coagulation abnormalities have been attributed to Hyperhomocysteinemia. Serum homocysteine was estimated on 5 ml of serum by competitive chemiluminescent enzyme immunoassay method and the specimen was transported to the laboratory within 30 minutes of collection. Results were assured as per standard quality control regime. The normal values of serum homocysteine in pregnancy during second trimester is 2-26.9 μmol/L, while it is 3.2-21.4 μmol/L in third trimester. Proportion of cases with normal level of serum homocysteine were observed in 37.5% and that controls were 87.5%. Proportion of cases with mild elevated levels of serum homocysteine were observed in 37.5% and that controls were 12.5%. Proportion of cases with moderate and marked elevated levels of serum homocysteine were observed in 17.5% and 7.5% respectively as compared to zero controls. This difference in the proportion of serum homocysteine levels amongst cases and controls was found statistically significant i.e. (p<0.05). It means serum homocysteine levels were significantly elevated in preeclampsia cases in our study.

Key words: Serum homocysteine, severe preeclampsia, term gestation

Introduction

Hypertensive disorders of pregnancy (HDPs) complicate 5–10% of all pregnancies and remain leading causes of maternal and perinatal morbidity and mortality worldwide [1]. Fetal/neonatal short-term complications include intra uterine growth restriction (IUGR), small for gestational age (SGA), low birth weight, preterm birth, and intra uterine and perinatal deaths [2].

A stillbirth rate of about 10% and a perinatal mortality rate of at least 5% have been reported in HDPs [3]. Acute-onset severe systolic hypertension (≥160 mmHg), severe diastolic hypertension (≥110 mm Hg); or both can occur during the prenatal, intra partum, or post partum periods. Preeclampsia with severe features in pregnancy is an emergency that requires urgent intervention [4]. HDPs are one of the most common indications for fetal surveillance aiming to reduce the burden of perinatal mortality and long-term morbidities, while also avoiding unnecessary obstetric interventions [5].

Preeclampsia (PE) is a multi-system pregnancy specific syndrome, which manifests by the onset of hypertension after 20 weeks of gestation in a previously normotensive pregnant woman and is believed to resolve with termination of pregnancy or by 12 weeks postpartum. As clinical presentation of PE is highly variable and even with severe disease a woman may

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be asymptomatic, the diagnosis is highly challenging [6]. If there is no timely detection of PE, it can progress to multi-organ failure, coagulopathy, maternal and fetal death in its severe form [7]. There are several predictors of PE, among which the evidence of high blood pressure values is the most reliable. Among bio- humoral parameters, homocysteine has recently been considered a possible remarkable cause of vascular damage [8]. Hyperhomocysteinemia is considered as a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis. Homocysteine mediated vascular changes are similar to those associated with PE and thus it has been hypothesized that hyperhomocysteinemia may be associated with PE [7]. It was also found to be associated with placenta-mediated diseases such as placental abruption and may lead to miscarriage, IUGR, LBW, prematurity and congenital malformations.

Homocysteine is an intermediate product of Methionine metabolism. As methionine cannot be stored in liver, it is demethylated to Homocysteine for storage purpose [9]. The total plasma Homocysteine level of healthy individual varies with age, gender, geographical areas and environmental factors. Normal values are between 3-7 μmol/L [10]. Hyperhomocysteinemia is defined as fasting plasma value more than 7 μmol/L. Hyperhomocysteinemia may be a because of deficiency of folic acid, vitamin B6 and B12 or genetic defect of enzyme required for Homocysteine metabolism [11].

Hyperhomocysteinemia has been known to cause many vascular diseases. But the exact pathogenesis of Hyperhomocysteinemia induced vascular damage is not clearly known. Several factors such as oxidative stress, endothelial dysfunction, smooth muscle cell proliferation and coagulation abnormalities have been attributed to Hyperhomocysteinemia [10]. These factors might lead to placental dysfunction leading to various pregnancy complications like recurrent pregnancy loss, pre- Eclampsia, Eclampsia, Abruptio placentae and Fetal growth restriction (FGR) with oligohydramnios [12]. Placental dysfunction might also lead to oligohydramnios without causing FGR.

Methodology

Study population

Group 1: Cases - pregnant women clinically diagnosed with severe preeclampsia in third trimester (BP>160/110, Proteinuria 3gm/day, with or without pathological edema)

Group 2: Control- normal pregnant women in third trimester

Study design: Case control study

Sample size: We planned to take 80 patients. We have two groups. Accordingly, in each group we included 40 subjects.

Sampling technique: Simple Random sampling method

Inclusion criteria

- Normotensive pregnant women in third trimester
- Pregnant women with severe preeclampsia at term
- singleton pregnancy
- No imminent signs

Exclusion criteria

- Pregnant Women with chronic hypertension, gestational hypertension, mild preeclampsia, eclampsia.

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- Pregnant women with conditions like diabetes mellitus, gestational diabetes, cardiovascular disease, chronic liver and kidney disease, severe anemia, multiple pregnancies and chronic diseases that interfere withstudies.
- Pregnant women with the history of smoking/alcoholism
- Pregnant women on antifolate drugs like methotrexate
- History of epilepsy in prepregnant state, space occupying lesion in brain.

Methods of data collection

We carried out a face-to-face interview using a pre-designed and pre-tested proforma. The proforma included information pertaining to the age, gestational age, obstetric score and the last menstrual period (LMP). The expected date of delivery (EDD) was calculated. Information regarding the marital history, menstrual history, significant past history, medical history, history of treatment taken for PE was included in the proforma. A comprehensive general physical examination, systemic and obstetric examination was conducted. BP was measured using a Mercury Sphygmomanometer in the right arm, sitting position. The BP measurement was repeated after 15-20 minutes and the highest reading of the two was entered in the proforma. The participant was asked to submit a
random midstream urine sample and proteinuria was estimated using a spot urine dipstick method, using visual reagent strips, considered as a quick, portable, and easy to do method for analysis of proteinuria. Serum homocysteine was estimated on 5 ml of serum by competitive chemiluminescent enzyme immunoassay method and the specimen was transported to the laboratory within 30 minutes of collection. Results were assured as per standard quality control regime. The normal values of serum homocysteine in pregnancy during second trimester is 2-26.9 μmol/L, while it is 3.2-21.4 μmol/L in third trimester.
Approval from the institutional ethics committee was obtained before conducting the study. Detailed information pertaining to the nature, objectives of the study and test procedures was provided to the study participants and written informed consent was obtained. Anonymity of the study participants was ensured. Strict confidentiality of the information collected was maintained.

Results

Table 1: Distribution of cases and controls according to serum homocysteine levels

Serum homocysteine	Cases		Controls		p
	Frequency	Percent	Frequency	Percent	
Normal	15	37.5	35	87.5	0.0001 Highly significant
Mid	15	37.5	5	12.5	
Moderate	7	17.5	0	0.0	
Marked	3	7.5	0	0.0	
Total	40	100.0	40	100.0	

Proportion of cases with normal level of serum homocysteine were observed in 37.5% and that controls were 87.5%. Proportion of cases with mild elevated levels of serum homocysteine were observed in 37.5% and that controls were 12.5%. Proportion of cases with moderate and marked elevated levels of serum homocysteine were observed in 17.5% and 7.5% respectively as compared to zero controls. This difference in the proportion of serum homocysteine levels amongst cases and controls was found statistically significant i.e. (p<0.05). It means serum homocysteine levels were significantly elevated in preeclampsia cases in our study.

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Table 2: Association of serum homocysteine with IUGR in cases and controls

		IUGR				p
		Cases		Controls		
		Frequency	Percent	Frequency	Percent	
Serum homocysteine	Normal	5	50.0	0	0.0	--
	Mid	3	30.0	0	0.0	
	Moderate	2	20.0	0	0.0	
	Marked	0	0.0	0	0.0	
Total		10	100.0	0	0.0	

Out of 10 IUGR babies born to preeclamptic mothers in our study half i.e., 50% had mild and moderately elevated levels of serum homocysteine. There is significant association between elevated levels of serum homocysteine and IUGR rate in our study.

Table 3: Association of serum homocysteine with LSCS in cases and controls

		LSCS				p
		Cases		Controls		
		Frequency	Percent	Frequency	Percent	
Serum homocysteine	Normal	6	35.3	18	85.7	0.0013 Highly significant
	Mid	7	41.2	3	14.3	
	Moderate	2	11.8	0	0.0	
	Marked	2	11.8	0	0.0	
Total		17	100.0	21	100.0	

Out of 17 babies born to preeclamptic mothers with LSCS as mode of delivery 41.2% had mild and 11.8% had moderately and markedly elevated levels of serum homocysteine. Out of 21 babies born to control mothers with LSCS as mode of delivery 14.3% had mild elevated levels of serum homocysteine. There is significant association between elevated levels of serum homocysteine and caesarean rate in our study.

Table 4: Association of serum homocysteine with NICU admission in cases and controls

		NICU admissions				P
		Cases		Controls		
		Frequency	Percent	Frequency	Percent	
Serum homocysteine	Normal	6	35.3	8	100.0	0.012 Significant
	Mid	6	35.3	0	0.0	
	Moderate	4	23.5	0	0.0	
	Marked	1	5.9	0	0.0	
Total		17	100.0	8	100.0	

Out of 17 babies born to preeclamptic mothers 6 i.e. 35.3% had mild elevated levels of serum homocysteine were observed. Out of 17 babies born to preeclamptic mothers 4 i.e. 23.5% had moderately elevated levels of serum homocysteine were observed. There is significant association between elevated levels of serum homocysteine and NICU admission rate in our study.

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Table 5: Association of serum homocysteine with fetal outcome in cases and controls

		Death of newborn baby				p
		Cases		Controls		
		Frequency	Percent	Frequency	Percent	
Serum homocysteine	Normal	2	40.0	2	100.0	0.8 Not significant
	Mid	3	60.0	0	0.0	
	Moderate	0	0.0	0	0.0	
	Marked	0	0.0	0	0.0	
Total		5	100.0	2	100.0	

Out of 5 new born deaths in preeclamptic group, 3 i.e. 60% had mild elevated levels of serum homocysteine were observed.

Discussion

Proportion of cases with normal level of serum homocysteine were observed in 37.5% and that controls were 87.5%.Proportion of cases with mild elevated levels of serum homocysteine were observed in 37.5% and that controls were 12.5%. Proportion of cases with moderate and marked elevated levels of serum homocysteine were observed in 17.5% and 7.5% respectively as compared to zero controls. This difference in the proportion of serum homocysteine levels amongst cases and controls was found statistically significant i.e. (p<0.05). It means serum homocysteine levels were significantly elevated in preeclampsia cases in our study.

The mean serum homocysteine levels in our study were found to be significantly higher among the preeclamptic women when compared to the normotensive women (p <0.001).

Homocysteine is an amino acid which has gained prominence in the recent years.

The mean homocysteine levels normally decrease with gestation either due to physiological response to the pregnancy, increase in estrogen, hemodilution from increased plasma volume or increased demand for methionine by both the mother and fetus.

However, it is known to be an offending factor in the vascular pathology causing PE.

Stoĭkova V *et al.* [13], have reported that homocysteinemia is an important independent cardiovascular risk factor, which might induce the endothelial dysfunction observed in preeclampsia.

Likewise, the role of elevated Serum Homocysteine in PE has been explored by several researchers in the past.

In the present study, a significant difference was found between the mean serum homocysteine levels of preeclamptic women and normotensive women (p <0.001).

The results of a research carried out by Wadhwani NS *et al.* [14], in Pune in the year 2015 was in concordance with this finding.

This finding was also similar to the result of a study carried out by Sangeetha N *et al.* [15], in New Delhi and Sanlikan F *et al.* [16], in Poland.

On the contrary, D' Anna R *et al.* [17] who conducted a study in a cohort of 1874 pregnant women, didn't report a statistically significant difference in the mean homocysteine among women with PE and normotensive controls.

Patil N. *et al.* [18] reported that mean Homocysteine level was more in study group (26.4mmol) compared to control group (10.2mmol) with a p value <0.05. Also in each pregnancy complication subgroup, the level was significantly more compared to the control group (p value <0.05).

Naga Jyothi S. *et al.* [19] reported that study group (diagnosed PE cases), 20 cases were mild PE, 25 cases were severe PE. The mean serum homocysteine levels in mild PE cases was 14.5±3.2µmol/l, which when compared to normotensive pregnant women is elevated and is highly statistically significant (p<0.001) which is consistent with our findings [20].

Conclusion

Present study concludes that preeclamptic women have a significantly higher serum

homocysteine when compared to normotensive women. Thus, the findings of this study stress that elevated serum homocysteine has a definite role to play in PE.

- Hyperhomocysteinemia appears to be more common among pregnant women with pre-eclamptic toxemia when compared to normotensive pregnant women of same gestational age suggesting that endothelial cell damage in these patients may be mediated by hyperhomocysteinemia

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