Original Research Article

Hyperhomocysteinemia in pre-eclampsia: is routine screening rational?

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ABSTRACT

Background: Hypertensive disorders complicate upto 5 to 10% of all pregnancies. Though the exact cause of pre-eclampsia is still undecided, maternal hyperhomocysteinemia has been implicated as a risk factor for pre eclampsia, placental abruption and other vascular diseases. The objectives of present study were to estimate the levels of serum homocysteine in antenatal patients and to study the above parameters in patients of pre-eclampsia.

Methods: A prospective observational study was performed with 30 pre eclamptic patients and an equal number taken as control having comparable demographic characteristics. Level of homocysteine was measured by an enzymatic method, using Diazyme homocysteine 2 reagent enzymatic assay kit on Beckman coulter analyzer in all the patients. Obstetrics and neonatal outcomes were observed in all the patients. The statistical analysis was done using unpaired T test for determining level of significance.

Results: Mean Serum homocysteine in the study group was 13.99 ± 5.46 µmol/l and was 6.03 ± 2.58 µmol/l in control group. This was statistically significant (p value 0.002). However the mean values of serum homocysteine did not correlate with severity of pre-eclampsia 14.32 ± 6.72 µmol/l in mild pre-eclampsia and 13.60 ± 3.77µmol/l in severe pre-eclampsia respectively (p value - 0.727).

Conclusions: It appears that maternal serum homocysteine has a causal role in pathogenesis of pre eclampsia, however to recommend it as a routine test, larger studies are required.

Keywords: Homocysteine, Pre-eclampsia, Pregnancy

INTRODUCTION

Hypertensive disorders complicate upto 5 to 10% of all pregnancies. Along with hemorrhage and infection, it forms the deadly triad that contributes to maternal morbidity and mortality.[1] In 2011-2012, 7.6% of maternal deaths were due to hypertensive disorders of pregnancy.[2] Though the exact cause of pre-eclampsia is still undecided, endothelial dysfunction with associated intense vasospasm has been implicated in its causation. Elevated maternal plasma levels of homocysteine (Hcy) have been recognized to play an important role in the etiology of pre-eclampsia and other vascular diseases. Hyperhomocysteinemia induces oxidative injury to endothelial cells which may trigger pathological consequences of pre-eclampsia.[3]

Though several studies support this hypothesis, yet no recommendations have been laid down regarding use of this test in routine clinical practice for screening patients who are at risk of developing hypertensive disorders of pregnancy. Therefore the objectives of this study are to investigate serum homocysteine levels in normal pregnancy and in patients of pre-eclampsia to asses any association and to justify its role in the battery of investigations.

METHODS

This case control study was conducted in the Department of Obstetrics and Gynaecology at Himalayan Institute of Medical Sciences (HIMS), Dehradun, India from November 2015 to October 2016. Pregnant women were

recruited from OPD and emergency with gestational age between 28- 40 weeks of gestation after obtaining written informed consent. This was approved by the institute ethical committee. Design of the study was prospective observational study. There were 60 subjects in sample size and sampling method.

Inclusion criteria

Primigravida or multigravida pregnancy with gestational age of 28-40 weeks.

- Study group: 30 pre-eclamptic pregnant women
- Control group: 30 normotensive pregnant women

Members of the study group were selected consecutively as and when they presented. Pre-eclampsia was diagnosed by blood pressure ≥140/90 mm of Hg on more than 2 occasions and proteinuria was measured by 300mg or more protein in a 24 hr urine collection or dipstick indicating 1+ or more than 1+(30 mg/dl or more than that) in random urine samples.

Exclusion criteria

- Gestational age other than 28-40 weeks.
- Pregnant women with: diabetes mellitus, chronic hypertension, neural tube defects and repeated miscarriage.

Study tools

- Case Recording Form.
- Reagents Homocystiene on Beckman Coulter Analyzer.

Study protocol

The detailed history (name, age, sex, occupation, residential address, chief complaints, history of presenting illness, obstetric history, past history, personal history, history of chronic illness, family history) was taken and clinical examination carried out as per the designed proforma in all patients.

Measurement of homocysteine

5ml of blood sample was drawn from the ante cubical vein and collected in the blood collecting tube. All the specimens were transported to the laboratory within 30 minutes of collection. Thereafter, specimens were centrifuged for 5-7 minutes at 3000 rpm. Then clear serum was transferred in a plastic vial and stored in refrigerator at -200°C until analysis.

The level of homocysteine was measured by an enzymatic method, using Diazyme homocysteine 2 reagent enzymatic assay kit on Beckman coulter analyzer after calibrating the machine and running standards. Normal range of serum homocysteine was taken as 3-13 µmol/l. Value ≥13 umol was taken as hyperhomocysteinemia.

Statistical analysis

A database was constituted using solutions (SPSS Version 22) and electronic spreadsheets (MS Excel) to store and manage the collected data. Qualitative data was expressed in term of frequency percentage and quantitative data expressed in terms of mean±SD. Student 't' test was used to compare the mean values of serum homocysteine between the groups. Chi-square test was used to compare the test of proportion between groups. P value <0.05 was considered as statistically significant. All the patients were followed till delivery for any obstetrical complications and neonatal outcome was also noted.

RESULTS

A total of 60 subjects were included in the study. 30 subjects were taken as study group (pre eclamptic women) and 30 patients were taken as control group (normotensive pregnant women). The patients were comparable in all respects. No patient was excluded after enrolment.

Table 1: patient characteristics in the two groups (n=60).

Parameters	Study group (n=30)		Control group (n=30)	
	No.	%	No.	%
Age Group				
20 - 25	19	63.33%	20	66.66%
26 - 30	7	23.33%	7	23.33%
> 30	4	13.33%	3	10%
Mean	24.93±3.17		24.93±3.97	
Gestational age				
28 - 30.6 weeks	5	16.66%	0	0%
31 - 36.6 weeks	13	43.33%	12	40%
> 37 weeks	12	40%	18	60%

Table 2: Mean of serum homocysteine levels in both the groups.

Variables	Study group (n=30)		Control group (n=30)		t-value	p -value
	Mean	Sd	Mean	Sd		
S. Homocysteine (umol/l)	13.99	5.46	6.03	2.58	7.206	0.002

Table 3: Levels of serum homocysteine in study group and control group.

S.Homocysteine (umol/l)	Study group (n=30) %	Control group (n=30) %	Chi square	p-value
< 3	0 (0%)	3 (10%)		
3-12.9	16 (53.33%)	27 (90%)	19.81	00
≥ 13	14 (46.66%)	0 (%)		

Table 4: Serum homocysteine levels in mild and severe pre-eclampsia (PE).

Severity of pre-eclampsia	No. (%)	S. homocysteine (µmol/l)		
		Range	Mean	SD
Mild PE	16(53.33%)	5.3-30.8	14.32	6.72
Severe PE	14(46.66%)	7.1-20.1	13.60	3.77
T value = 0.353, P value = 0.727				

Table 5: Mean serum homocysteine of patients with prematurity and IUGR.

	Prematurity	IUGR	p-value
Serum homocysteiene µmol/l	13.53±4.06	13.08±3.87	0.796

On comparison, the p value was not significant. Study group showed 56.66% of LSCS and control group had 90% of NVD 's.

DISCUSSION

Elevated plasma homocysteine concentration has been an independent risk factor for vascular diseases like pre-eclampsia and placental abrution in pregnancy and other artherogenic conditions such as coronary artery diseases. Endothelial cell dysfunction has been postulated as the recent hypothesis of pre-eclampsia and raised plasma homocysteine concentration may contribute to this pathology. Maternal characteristics are depicted in Table 1 this matches with the observations by Premlata Mital et al.[4] The mean age in their study was 24.55±3.4 years, and 24.00±4.41 as studied by Md.Mozammel Hoque et al.[5] In contrast khosrowbeygi A Ahmadvand H showed a higher mean age of pre eclamptic group as (32.27±0.69).[6]

The mean serum homocysteine levels in our study in study group were found to be 13.99±5.46 (µmol/l) and control group 6.03±2.58 (µmol/l), which was statistically significant (p value-0.002). Arpita p patel found mean value of homocysteine in control group and study group to be 13.45±4.40 µmol/l vs 19.96 umol/l ± 6.42±2.58 µmol/l with (p value -0.002).[7]

Several other studies were in consonance with the present study.[5,6,8-10] On the contrary Zeeman GG et al did not agree with our observations and there was no significant difference in maternal serum levels of homocysteine between pre-eclampsia and normal pregnancy (5.1±1.7 µmol/l vs 4.7±1.3 µmol/l p value =0.56) and they stated that serum homocysteine levels were not helpful in the prediction of PE.[11] Hietala R et al also stated that there was no difference between pre-eclampsia and normotensive women (6.42, 7.55µmol/l vs 6.45, 7.34 µmol/l).[12] We also compared mean serum homocysteine in mild and severe pre-eclampsia (14.32±6.72vs13.60±3.77µmol/l respectively) p value-0.727 which was statistically not significant. This indicates that serum homocysteine could not be used as a marker for predicting the severity of pre eclampsia. Our results are also supported by Faith Şanlıkan et al.[13] On the contrary several studies have showed that levels of serum homocysteine were higher in severe pre eclampsia. Premlata et al showed the mean levels of severe PE and mild PE 15.9±6.3 vs 12.3±4.2 (p value =0.01).[4] Several other studies were in consonance with the her results such as Khosrowbeygi A et al and Makedos G et al.[6,10]

Based on the findings of present study it can be concluded that maternal serum homocysteine seems to have a causal role in pathogenesis of pre-eclampsia, however measurement of homocysteine concentration did not determine the severity of pre-eclampsia. In present study the pre-eclamptic group had higher incidence of caesarean sections (56.66%). 17 out of 30 patients had LSCS in the study the main indications were fetal distress IUGR, oligohydraomnios, non progress of labour, this clearly highlights the rising trend caesarean delivery in high risk pregnancies like severe pre-eclampsia IUGR, oligohydroamnios which could be prevented well in time with appropriate tools. Similar results were reported by Georgios Makedos et al.[10] Increased homocysteine is a risk factor for IUGR which was found in our study too (Table 6). 30% subjects in our study group (9 out of 30) had IUGR. Serum homocysteine levels were found higher to normal in these patients the mean S.hcy were (13.08±3.87 µmol/l). Our observation was similar to seema Bibi et al.[14]

In this study 46.66% patient in study group had preterm delivery, the serum levels of hcy were 13.53±4.06 µmol/l. Babies in the study group were delivered prematurely because of induced delivery done for severe pre eclampsia. In our study 3 patients had IUFD due to severe IUGR and oligohydramnios in cases of severe pre-eclampsia and acute placental abruption, 2 babies were still born in mothers who presented with massive abruption it again reflects the neonatal morbidity, need for neonatal ICU care and burden on the patient and health care system in patients of hypertensive disorders of pregnancy.

Therefore, it is imperative to identify the pregnant women who are at risk of developing pre-eclampsia. Though hyperhomocysteinemia seems to be implicated in the etiopathogenesis of pre-eclampsia yet to advocate it as a necessary initial investigation is debatable. Considering high cost of investigation, serum homocysteine could complement to other screening modalities for pre-eclampsia and be judiciously offered to those cases which are at high risk of developing hypertensive disorders of pregnancy.

Table 6: Perinatal outcomes in different groups.

Perinatal outcome	Study group (%)	Mean S. Hcy (µmol/l)	Control group (%)	Mean S. Hcy (µmol/l)
Healthy	15 (50)	14.68	26 (86.66)	5.78
IUD	3 (10)	13.13		
Still birth	2 (6.66)	11.35		
Prematurity	14 (46.66)	13.53	4 (13.33)	3.92

CONCLUSION

Many prospective and retrospective clinical and epidemiological studies have proven that hyperhomocysteinemia is artherogenic and causes pregnancy related complications such as hypertensive disorders of pregnancy and poor neonatal outcome. This is valuable test which could be incorporated in the battery of investigations for hypertensive disorders of pregnancy. However more studies on a larger sample size, multicentric are required. Finally timed detection of cases with Hyperhomocysteinemia and its rectification by supplementing essential vitamins would go a long way in bringing down maternal and neonatal morbidity.

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