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Original research article

A prospective study of serum homocysteine in pregnancy related hypertensive disorders versus normotensive pregnancies in a tertiary care institute

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Abstract

The present study is a hospital based prospective study conducted in the Department of Obstetrics and Gynaecology. Two hundred patients (100 women with pregnancy related hypertensive disorders and 100 normotensive pregnant women) are recruited into this study. Maternal serum Homocysteine levels were analyzed among women with pregnancy related hypertensive disorders and compared with normotensive pregnant women. Chemiluminescence immunoassay was used for determining the Homocysteine levels. Statistical analysis was done and data was analysed. The present study revealed statistically significant elevation in the levels of Homocysteine in pre eclamptic women (17.54±5.34) compared to normotensive women (7.59±1.91). The study showed a strong association between serum Homocysteine levels and severity of pre eclampsia. The mean homocysteine levels in women with severe Pre eclampsia (20.59±4.3) were significantly higher than those with mild pre eclampsia (15.17±1.9). A negative correlation is found between Homocysteine levels with hemoglobin and hematocrit even though in anemic patients the hematological levels are found to be little high due to hemoconcentration. The perinatal outcome in women with raised levels of Homocysteine was poor with an increased incidence of IUGR (41%), SGA (9%), still births (3%) and IUD (4%) among women with pregnancy related hypertensive disorders when compared to normotensive pregnant women.

Keywords: Pre eclampsia, homocysteine, normotensive, hypertensive disorders

Introduction

Hypertensive disorders complicate 5 to 10% of all pregnancies [1]. Along with haemorrhage and infection, it forms the deadly triad that contributes to maternal morbidity and mortality [2]. Incidence of PIH in India ranges from 5% to 15%. PIH is associated with 16% of all maternal mortality & 20% of all perinatal mortality in India.

According to epidemiological evidence women with previous gestational hypertension seem to be at increased risk of cardiovascular disease, hypertension, stroke and death from ischaemic heart disease in later life compared with the general population. PIH develops due to pregnancy and regresses after delivery. It is a major cause of premature delivery, intrauterine growth restriction (IUGR), placental abruption, fetal death and numerous adverse pregnancy outcomes.

Though the exact cause of pre-eclampsia is still undecided, endothelial dysfunction with associated intense vasospasm has been implicated in its causation [2]. Homocysteine, a sulphur containing amino acid usually decreases in 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 [3]. Homocysteine is involved in processes such as lipid peroxidation, oxidative stress and endothelial dysfunction and thereby resulting in pre-eclampsia [3].

The present study is aimed at the estimation of serum homocysteine concentration in both pregnancy related hypertensive disorders and normotensive pregnant women, thereby deducing its relation in causation of pre-eclampsia.

Elevated homocysteine levels confer an independent and incremental risk for vascular disease, direct endothelial toxicity, failure of nitric oxide release and platelet abnormalities. Homocysteine may prove to be the missing link in the etiology of pre- eclampsia [3]. Further, hyperhomocysteinemia may also be an

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important biological marker for adverse outcome of pregnancy and even possibly a cause of or a contributor to the complications of pregnancy [4].
Current study aimed to compare maternal serum Homocysteine levels among normotensive pregnant women and those with pregnancy related hypertensive disorders.

Material and Methods
This is a prospective case-control study done in the Department of Obstetrics and Gynaecology. This study is to analyse the serum levels of homocysteine among pregnant women with pregnancy related hypertensive disorders and to compare with those of normotensive pregnancies.

Type of Study: Prospective case control study.
Sample Size: 200 pregnant women with 100 in each group A and group B.
Group A-cases: Pregnant women diagnosed with pregnancy related hypertensive disorders.
Group B-controls: Normotensive pregnant women.

Inclusion Criteria
- Pregnant women with pregnancy related hypertensive disorders (gestation hypertension, pre-eclampsia, eclampsia and chronic hypertension)
- Normotensive pregnant women, both primigravida and multigravida with no bad obstetric history

Exclusion Criteria
- Pregnant women with H/O smoking and alcoholism.
- Pregnant women with other conditions like gestational diabetes, diabetes mellitus, cardiovascular disease, chronic liver and kidney disease, anemia, multiple pregnancies and other chronic diseases that interfere with the study.
- Pregnant women on antioxidant like vitamin E.

Patient Analysis
No bias when sample selection was done with respect to age, parity, socioeconomic status.
1. A written consent was taken from all subjects after explaining them, regarding the study and then they were included in the study.
2. The study group should not be exposed to any increased risk as a result of the study.
3. Confidentiality should be maintained.

All the women with pregnancy related hypertensive disorders and normotensive pregnant women attending the obstetrics department were recruited into the study and control groups following detailed history and clinical examination. Apart from routine antenatal investigations specific investigations were sent related to the present study. In the present study serum Homocysteine levels were measured in both study and control groups.

Methodology
Blood samples were collected preferably from the antecubital vein irrespective of fasting status. In cases, sample was taken after the diagnosis of pre-eclampsia or eclampsia was made immediately after admission and before initiation of antihypertensive treatment and before the delivery. All the specimens were transported to the laboratory within 30minutes of collection. After that, specimens were centrifuged for 5-7 minutes at 3000rpm. Then clear serum was transfused in aplastic vial and stored in refrigerator until analysis. Samples were stored at 2-8 ºC. Chemiluminescence immunoassay was used for determining the Homocysteine levels. The system used was an automated, random access, direct CLIA analyser.

Results
One hundred pregnant women fulfilling the inclusion criteria were recruited in the study group. Among the group 65 were primigravida and 35 were multiparous women. Another 100 normal pregnant women were taken as control group among whom 75 were primigravida and 25 were multiparous women.
In the present study, the age distribution varied from 19-35 years. The majority of pregnant women 46% in study group and 51% in control group were under the age group of 25-30years. 15% of women in study group and 16% of women in control group were elderly gravida with age 30-35years.
The mean age among the pregnant women with pregnancy related hypertensive disorder (study group) is 25.6yrs and 25.93yrs in normotensive pregnant women (control group) which is not significant.
The mean gestation age in women is 34.88 weeks in study group and 34.81 in control group. This shows there is no significant difference among the women with pregnancy related hypertensive disorders and normotensive pregnant women with regard to the period of gestation.
The mean DBP in study group is 97.2±8.1 mm of Hg and in control group is 73.57±6.7 mm of Hg with a

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significant p value of <0.0001 as shown by the above table and figure.
The above values indicate that there is a rise in the DBP among the PIH cases when compared to the normal pregnant women.
The mean arterial pressure (MAP) in the study group is 115.35±6.9 mm of Hg and 86.4±4.9 in the control group with a significant p value <0.001.

Table 1: Mean Serum Homocysteine-Study vs. Controls

	Study	Control	p Value
S. Homocysteine (µmol/L)	17.54±5.34	7.59±1.91	<0.0001(HS)

The mean serum homocysteine levels in women with pregnancy related hypertensive disorders (study group) is 17.54±5.34µmol/L and in normotensive pregnant women is 7.59±1.91µmol/L with a significant p value <0.0001.

Table 2: S. Homocysteine Levels and SBP in Study Group

SBP (mmHg)	n	Mean S. Homocysteine (µmol/L)
140-149	28	15.33±4.27
150-159	35	16.38±5.30
160-169	36	20.30±5.03

Table 3: S. Homocysteine Levels and DBP: Study Group

DBP (mmhg)	n	Mean S. Homocysteine (µmol/L)
90-100	49	15.24±4.02
101-109	29	17.51±0.78
110-119	22	22.68±1.24

Table 4: Serum Homocysteine Levels and Mean Arterial Pressure (MAP): Study Group

MAP (mmHg)	n	Mean S. Homocysteine (µmol/L)
100-109	17	13.93±2.5
110-119	48	15.70±3.7
120-129	33	21.45±5.4
130-139	2	27.65±2.7

Table 5: Serum Homocysteine in Pregnancy Related Hypertensive Disorders

Diagnosis	N	Mean S Homocysteine (µmol/L)
Chronic hypertension	5	13.28 ± 2.84
Gestational hypertension	17	14.63 ± 3.04
Pre eclampsia	67	17.04 ± 3.91
Eclampsia	11	27.05 ± 6.26

Table 6: Comparision Serum Homocysteine and Hemoglobin, Hematocrit in Study vs. Controls

	HB (g/dl)	PCV (vol. %)	Mean S. Homocysteine (µmol/L)
Study Group	9.54±1.15	31.55±3.37	17.54±5.34
Control Group	9.97±1.32	32.54±3.57	7.59±1.91
P Value	0.015 SG	0.045 SG	<0.0001 HS

Table 7: Mode of Delivery-Study vs. Controls

Mode of Delivery	Study Group	Control Group	Total
Caesarean Section	27	14	41
Vaginal Delivery	73	86	159
Total	100	100	200

Table 8: S. Homocysteine and Perinatal Outcome: Study vs. Controls

Perinatal Outcome	Study Group (n)	Mean Homocysteine (µmol/L)	Control Group (n)	Mean Homocysteine (µmol/L)
Normal Growth	43	16.23±3.67	94	7.61±1.90
SGA	9	16.84±3.61	4	8.08±2.55
IUGR	41	17.34±5.32	2	6.15±1.77
IUD	4	29.2±6.38	0	
Still Birth	3	25.53±6.11	0	

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Discussion

Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality. Although, the definitive etiology of pre-eclampsia is still unknown, the basic pathology involved is endothelial dysfunction and intense vasospasm. Homocysteine, a metabolite of essential amino acid Methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction thus contributing as an etiological factor of pre eclampsia [3].

Serum Homocysteine appears to be the missing link in the etiology of preeclampsia [3].

Hyperhomocysteinemia has been reported to cause endothelial dysfunction by scavenging or trapping nitric oxide. Nitric oxide (NO) plays an important role in mediating the cardiovascular and hemodynamic changes during pregnancy. In addition, a decrease in nitric oxide production and availability has been shown to increase Endothelin production, reduce the renal pressure natriuresis relationship, causing hypertension. Mean arterial pressure is useful concept because it can be used to calculate overall blood flow and thus delivery of nutrients to various organs. It is a good indicator of perfusion pressure.

The present study is in accord with the above studies showing raise of MAP and serum Homocysteine levels in women with pregnancy related hypertensive disorders compared to normotensive pregnant women with a significant p value.

The studies done by SEEMA BIBI *et al*., and R NOTO *et al*., have shown a positive correlation between serum Homocysteine levels and MAP with a significant p vaue. The present study shows a positive correlation with significant p value ($r= 0.832$ $p=<0.0001$) agreeing with the above studies [6-10].

Elevated circulating of Homocysteine is a risk factor of endothelial dysfunction and vascular diseases such as atherosclerosis and occlusive disorders. The vascular effects of hyperhomocysteinemia have been proposed to include endothelial cell injury and thrombus formation. Maternal hyperhomocysteinemia is associated with a number of placenta-mediated diseases such as preeclampsia and eclampsia. Homocysteine appears to be the missing link in etiology of preeclampsia [3].

The present study findings are in par with the above studies showing higher serum Homocysteine values in severe pre eclampsia (20.59±4.4) compared to mild pre eclampsia (15.17±1.90) with a significant p value<0.0001.

Serum creatinine concentrations were significantly higher in pre eclamptic women as compared to controls. This is probably due to reduced renal perfusion and glomerular filtration rate, secondarily to abnormal glomerular morphology, i.e., endotheliosis in pre eclampsia. Since nearly 70% of Homocysteine elimination is dependant on renal uptake and metabolism of Homocysteine, altered renal function might be responsible for hyperhomocystenemia in pre eclampsia [11]. Raised serum uric acid levels indicate renal involvement by increased tubular reabsorption, decreased tubular secretion and diminished renal flow. High level of serum uric acid is found to correlate with the severity of pre eclampsia, volume contraction and fetal jeopardy.

Uric acid impairs nitric oxide generation in the endothelial cells inducing endothelial dysfunction. Besides the reduced clearance, hyperuricemia in pre-eclampsia may be due to increased uric acid production caused by trophoblast breakdown, cytokine release and ischemia. Uric acid can cause endothelial dysfunction, damage and inflammation, which leads to oxidation. Elevated circulating Homocysteine is also a risk factor for endothelial dysfunction and vascular diseases such as atherosclerosis and occlusive vascular disorders [12].

In the present study the finding is consistent with raised LDH level in study group (550.33±132.36) compared to control group (370.50±106.92 with a significant 'p' value <0.0001.

To best of our knowledge, no studies were found comparing serum Homocysteine levels with serum LDH levels in women with pregnancy related hypertensive disorders.

The studies conducted by SEEMA BIBI *et al* shown a positive correlation between microalbuminuria and serum Homocysteine levels in pre eclamptic women. The present study also shows positive correlation in par with above studies with a significant p value.

Homocysteine is a potent excitatory neurotransmitter that binds to the N-methyl-D-aspartate (NMDA) receptor and also leads to oxidative stress, cytoplasmic calcium influx, cellular apoptosis, and endothelial dysfunction. There are a lot of researches which put in evidence that oxidative stress in utero-placental tissues with an important role in the development of placental-related diseases. Few authors have pointed that hypoxia is necessary to maintain stem cells in a fully pluripotent state. Physiological level of reactive oxygen species regulates the transcription factors. In normal pregnancies, the earliest stages of the fetus development take place in physiological hypoxia. This protects the developing fetus against the deleterious and teratogenic effects of reactive oxygen species (ROS).

Hemoglobin concentration and hematocrit (PCV) value rise both in mild and severe preeclampsia in comparison to normal pregnant women. The lower hematocrit of normal pregnancy is associated with a decreased oxygen capacity but with an increased oxygen delivery. In cases with proteinuric hypertension, a hematocrit above normal is associated with reduced systemic oxygen transport rate.

In the present study, there is a significant rise in Hemoglobin and Haematocrit values among the two groups because of resulting hemoconcentration.

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Conclusion

Elevated Homocysteine concentration injures the vascular endothelium, thereby contributing to the pathology of pre eclampsia. Elevated levels of Homocysteine can be reduced by administering vitamins which help by increasing the metabolism of Homocysteine. The internationally accepted treatment for hyperhomocystenemia is using a combination of folic acid 400 µg, vitamin B12 of 500 µg and pyridoxine 10mg initiating from pre-conceptional period, as these deficiencies are very often seen in our population.

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