National Journal of Basic Medical Sciences | ORIGINAL ARTICLE | Volume - IV, Issue-3

# HOMOCYSTEINE IN PREGNANCY INDUCED HYPERTENSION

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**ABSTRACT**

**Background:** Homocysteine (Hcy) an amino acid when it is increased causes Hyperhomocysteinemia and is associated with pregnancy induced hypertension (PIH), placental abruption, thromboembolic events, placental infarction and neural tube defects. This created the interest to analyze the levels of Hcy correlating it with the hypertension in pregnancy induced hypertensive patients. **Aim:** The aim of this study is to analyze, correlate and compare the plasma Hcy levels along with other routine biochemical parameters in normal pregnant and PIH and to arrive at a cut off level of Hcy for these two groups. **Materials and Methods:** The study population includes 140 subjects in age group of 20-40 years. Out of that 40 non-pregnant females were included in control group and in Study group 100 pregnant women in third trimester were included and sub grouped as: (1) 40 normotensive, (2) 60 PIH women. Hcy, fibrinogen and routine parameters were estimated and compared by Student's *t*-test. **Results:** The mean reference value of the plasma Hcy level was 5.75 ± 3.34 µmol/l it ranges up to 15 µmol/L. The value is same for control and normal pregnant women. The mean Hcy value was increased in the PIH women (17.73 ± 7.09) and there was an associated significant increase in cholesterol and triglyceride (TGL), along with mild increase in serum alanine transaminase and uric acid levels. **Conclusion:** The Hcy level is well above the reference range in the PIH women along with significant increase in cholesterol and TGL level. This is injurious to the endothelial cell and impairs the function leading to hypertension. And mean cut off value was arrived to differentiate between the two groups and so Hcy plays an important biomarker in maternal health.

**Key words:** Homocysteine, Hyperhomocystiemia, Pegnancy induced hypertension, Serum alanine transaminase

## INTRODUCTION

Hypertension disorders complicating pregnancy are common and form one of the deadly triad with hemorrhage and infection that results in a large number of maternal death. Homocysteine (Hcy) is a naturally occurring aminoacid derivative in the body. It is formed from demethylation of methionine consumed in diet. Our body cannot store methionine, so it is transported to the liver and is demethylated to Hcy for storage until needed. Hcy is required in several reactions that occur in the cell. About 50% is transsulfurated to cysthathionine, a source of cysteine. Most of the Hcy is cleared from plasma due to its metabolism in the kidney and due to urinary excretion[1] hence the liver play vital roles in Hcy metabolism. Hcy can reform its parent aminoacid methionine by way of methylation through methylcobalamine in a reaction requiring Hcy methyl transferase or from betaine a methyl donor, by a reaction catalyzed by betaine-Hcy methyl transferase.[2] Very little of Hcy i.e. nearly 1% is excreted in urine.[3] Hcy level as specified in reputed books or journals are as <15 µmol/L[4] or 5-15 µmol/L[5] nutritional disorder that impair Hcy metabolism include deficiency of methylcobalamin, folate and pyridoxine because the denovo synthesis of methionine methyl groups require the above as cofactor. The concentration of plasma Hcy normally decreases during pregnancy as per Power *et al*. it was 7.0 ± 2.3 µmol/L.[6] Increase in hormones such as estrogen and cortisol during pregnancy may also mediate this specific decrease in Hcy concentration.[6]

Hyperhomocysteinemia (HHcy) is common with coronary artery disease, cerebral and peripheral vascular disease and renal dysnfunction. As per Jian Wang the maternal syndrome of preeclampsia and the fetal syndrome of intrauterine growth restriction have been associated with vascular disease in the maternal utero-placental and fetal umbilical placental circulations; this suggested placental insufficiency and HHcy was found related to it[7] on comparison of the upper with the lower quartile of plasma total Hcy the adjusted risk for preeclampsia was found to be 32% higher, that for prematurity was 38% higher, that for very low birth weight was 100% higher than in uncomplicated normal pregnant women.[8] The mechanism behind the vascular effect of Hcy namely platelet abnormalities stimulated coagulation or inhibited fibrinolysis, smooth muscle proliferation and endothelial dysfunction had been demonstrated in experiments[9] in general, the development and

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progression of atherosclerosis is considered to be a form of chronic inflammation.[10-13] Hcy induced expression of monocyte chemoattractant protein I is known to enchance monocyte endothelial binding and recruitment to the formation of fatty streaks.[13] Hcy is also been shown to increase expression of interleukin-8 neutrophil chemo attractant[14] patient suffering from HHcy develop extensive arterial intimal thickening and fibrous plaques rich in smooth muscles and collagen.[9,15]

Likewise Powers *et al*. obtained elevated plasma Hcy level in preeclamptic group as against the pregnant control.[6] He attributed the HHcy in preeclampsia to a deficit in the mechanism it usually decreases the Hcy levels in normal pregnancy. He also correlated with other biochemical parameters namely folic acid, triglyceride (TGL), creatinine and uric acid concentrations. Hcy did not correlate with plasma creatinine or TGL concentration and folate concentration weakly but negatively correlated with Hcy.[6]

In the work of Eva Lopez–Quesada *et al*. along with increase of Hcy in preclampsia there was increases in both essential and nonessential aminoacid.[16] All the above findings show that induced atherogenic alterations probably start to take place much earlier in the course of Preeclampsia and they develop hypertension, edema, proteinuria and other symptoms. So, early detection can prevent the patient from further deadly complications.

## MATERIALS AND METHODS

Cross-sectional study of 140 female subjects were chosen in the age group of 20-40 years. Out of them 40 were non-pregnant who formed control group. Forty normotensive pregnant women in their third trimester were chosen as Group 1. Sixty pregnancy induced hypertensive patients in their third trimester were chosen as study Group 2. As eclampsia cases were not available as of response to treatment in pregnancy induced hypertension (PIH) patients the study could not be conducted in such a group.

### Inclusion criteria

*Control group Normal non-pregnant women*

Study group Group 1

1. Normotensive pregnant women in their third trimester

Pregnant women with pregnancy induced hypertension (study Group 2)

Pregnant women in their third trimester with:

1. Persistent elevation of blood pressure 140/90 mmHg and more are confirmed by 2 measurements (in the sitting posture at least 6 h apart) or an increase of at least 30 mm of Hg systolic or 15 mm of Hg diastolic above base line value
2. Proteinuria - trace, 1+, 2+ or more by dipstick method.

### Exclusion criteria

Women with previous history of hypertension, diabetes, renal or heart diseases and other complications of pregnancy were excluded.

A fasting venous blood sample was collected with strict aseptic precautions.

Homocysteine level, fibrinogen, and total cholesterol, TGL, total protein, urea, albumin, serum glutamic-oxaloacetic transaminase, serum alanine transaminase (SGPT) creatinine, glucose and uric acid levels were calculated.

### Estimation of homocysteine

It was done by Axis Hcy enzyme immunoassay using enzyme-linked immunosorbent assay method.

### Principle

Axis Hcy is an enzyme immunoassay for the determination of total Hcy in blood. Protein bound Hcy is reduced to free Hcy and enzymatically converted to S-adenosyl-L-Hcy in a separate procedure prior to the immunoassay. The enzyme is specific for the L-form of Hcy which is the only form present in the blood.

The following solid phase enzyme immunoassay is based on competition between SAH in the sample and immobilized SAH bound to the walls of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. After the removal of anti-SAH antibody not bound to the plate, a secondary rabbit anti-mouse antibody labeled with the enzyme Horse Radish peroxidase [HPR] is added. The peroxidase activity is measured spectrophotometrically after addition of substrate, and the absorbance is read in 450 nm within 15 min and is inversely related to the concentration of total Hcy in the sample. Reference range is 5-15 µmol/L.

The Student's *t*-test was used to compare the levels of the biochemical parameters analysed between the groups.

## RESULTS

The mean value of Hcy was 5.75 ± 3.34 µmol/L in the control group and study Group 1. There was significant increase in study Group 2 mean value of 17.73 ± 7.09 µmol/L $P \leq 0.001$ [Table 1]. The mean cut off value to differentiate between the two groups was arrived at after determining the sensitivity, specificity, +ve predictive and −ve predictive value given in Table 2.

There is a highly significant difference between the mean values of total cholesterol in the control and study Group 1 (169.42 ± 14. mg/dl) and the study Group 2 (219.11 ± 37.79 mg/dl). The TGL level is significantly increased in the study Group 2 $P \leq 0.001$. The comparison of the mean values of fibrinogen there was significant decrease in study Group 2 ($P < 0.001$).

Among the other parameters measured, uric acid and creatinine levels are significantly increased with the $P < 0.001$, while SGPT had moderately significant (MS) increase ($P < 0.004$) in Group 2 [Tables 3 and 4].

## DISCUSSION

The Hcy level is increased in the pregnancy induced hypertensive women (Group 2) compared to pregnant normotensive women (Group 1). The association of HHcy to PIH can be proclaimed to be due to some deficit in the mechanism that converts routinely Hcy to either methionine or cystathionine; the resulting increase in Hcy can increase the endothelial activation leading to Hypertension (HT).[17] Moreover in HT as the liver may be affected due to peripheral hemorrhagic necrosis the functions of the liver will be increased leading to increase in enzymes depicting liver functions; this is evidenced in our study by the MS increase of SPGT even though in the study group is still within the reference range. Hence it can be said that the association of HHcy with increasing BP in PIH is a vicious cycle.

The highly significant increase of cholesterol and TGL in the group of PIH women can be the result of unfolded protein response by Hcy which promotes the overproduction of endoplasmic reticulum lipid components resulting in their increase. As Hcy statistically differed from normal pregnant women and PIH, it was decided to arrive at a cut-off level of Hcy to differentiate the condition, after determining the sensitivity, specificity, +ve predictive value and −ve predictive value for the different cut off levels selected [Table 2].

Hence concentration of Hcy of 12 µmol/L and above suggested PIH [Figure 1]. From the discussion held so far it is clear that Hcy level can be used as a biochemical marker for PIH and preeclampsia.

**Table 1: Homocystiene levels**

| Group | Homocysteine level (mean±SD) | *P* value and significance |
|---|---|---|
| Normal non-pregnant women | 5.75±8.34 | 0.99 NS |
| Normal pregnant women | 5.86±3.04 | 0.99 NS |
| Pregnancy induced hypertensive women | 17.73±7.09 | 0.001 HS |

NS: Non-significant, HS: Highly sensitive, SD: Standard deviation

**Table 2: Homocysteine levels in pregnancy induced hypertension**

| Cut off value | Sensitivity | Specificity | +ve predictive value | −ve predictive value |
|---|---|---|---|---|
| 14 | 96.55 | 48.38 | 63.63 | 93.75 |
| 14.2 | 86.20 | 64.51 | 69.44 | 83.33 |
| 14.5 | 79.31 | 83.87 | 82.14 | 81.25 |
| 14.8 | 79.31 | 83.87 | 82.14 | 81.25 |
| 15 | 79.31 | 93.54 | 92 | 82.85 |
| 16 | 68.96 | 100 | 100 | 77.5 |

## CONCLUSION

From the study held on plasma Hcy and other biochemical markers analyzed on 140 subjects grouped into normal non-pregnant women, normal pregnant women and PIH the following inference have been obtained.

1. The reference range of Hcy in the study is 5.75 ± 3.34 µmol/L.
2. In PIH, whether it be mere hypertension or preeclampsia, Hcy is increased well above the reference range of the study.

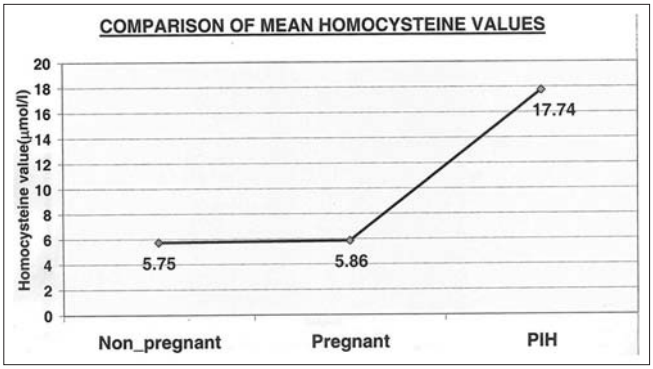


**Figure 1:** Comparison of mean homocysteine values

**Table 3: Mean and standard deviation of the biochemical parameters analysed in NNPW, NPW and PIH**

| | Hcy µl/l | Fibrinogen mg/dl | Sugar mg/dl | Urea mg/dl | Creatinine mg/dl | Uric acid mg/dl | Cholesterol mg/dl | TGL mg/dl | Total protein g/dl | Albumin g/dl | SGOT IU/L | SGPT IU/L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NNPW mean | 5.75 | 258.82 | 81.85 | 18.32 | 0.725 | 3.12 | 169.42 | 82.35 | 6.19 | 3.42 | 18.26 | 16.12 |
| SD | 3.34 | 42.24 | 9.58 | 3.91 | 0.125 | 1.12 | 14.61 | 15.63 | 0.5 | 0.19 | 4.67 | 3.48 |
| NPW mean | 5.86 | 262.05 | 81.85 | 18.32 | 0.873 | 2.47 | 169.42 | 87.4 | 6.44 | 3.44 | 18.65 | 19.42 |
| SD | 3.04 | 43.69 | 9.58 | 3.91 | 0.173 | 0.6 | 14.61 | 20.26 | 0.66 | 0.06 | 5.2 | 6 |
| PIHW mean | 17.73 | 233.65 | 89.76 | 20.23 | 0.92 | 4.39 | 219.11 | 155.33 | 6.01 | 3.45 | 19.73 | 19.95 |
| SD | 7.09 | 20.65 | 28.47 | 6.89 | 0.356 | 0.86 | 37.79 | 4.62 | 0.8 | 0.49 | 4.92 | 6.58 |

NNPW: Normal non-pregnant women, SD: Standard deviation, PIHW: Pregnancy induced hypertensive women, TGL: Triglyceride

**Table 4: Mean and standard deviation of the biochemical parameters analysed in NPW vs PIHW**

| | Hcy µl/l | Fibrin ogen mg/dl | Sugar mg/dl | Urea mg/dl | Creatinine mg/dl | Uric acid mg/dl | Cholesterol mg/dl | TGL mg/dl | Total protein g/dl | Albumin g/dl | SGOT IU/l | SGPT IU/l |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPW mean | 5.86 | 262.05 | 81.85 | 18.32 | 0.873 | 2.47 | 169.42 | 87.4 | 6.44 | 3.44 | 18.65 | 19.42 |
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| PIHW mean | 17.73 | 233.65 | 89.76 | 20.23 | 0.92 | 4.39 | 219.11 | 155.33 | 6.101 | 3.45 | 19.73 | 19.95 |
| SD | 7.09 | 23.65 | 28.47 | 6.89 | 0.356 | 0.869 | 37.79 | 4.62 | 0.8 | 0.49 | 4.92 | 6.58 |
| *P* value signi | 0.001 HS | 0.001 HS | 0.17 NS | 0.26 NS | 1 NS | 0.001 HS | 0.001 HS | 0.001 HS | 0.006 HS | 0.99 NS | 0.85 NS | 1 NS |

NPW: Normal pregnant women, SD: Standard deviation, PIHW: Pregnancy induced hypertensive women, TGL: Triglyceride, NS: Non significant, HS: Highly sensitive

3. In PIH, serum levels of creatinine, uric acid, cholesterol, TGL and SGPT are elevated; however only cholesterol and TGL are increased beyond their respective reference range.
4. Homocysteine concentration can act as biochemical marker to differentiate PIH from normal pregnant women and preeclampsia from mere PIH.
5. The determined cut-of level of Hcy differentiating PIH from normal pregnancy is 12 µmol/L.

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**Received:** 20.02.2014; **Accepted:** 11.04.2014