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Research Article

Association of maternal serum homocysteine level with severity of preeclampsia: a case control study

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ABSTRACT

Background: The objective of the study was to investigate plasma levels of homocysteine in women with preeclampsia and eclampsia, and to assess whether there is any association between hyperhomocysteinemia and the severity of preeclampsia.

Methods: In this case control study, 120 women were recruited in the study in which 40 cases of preeclampsia, 40 cases of eclampsia and 40 healthy normotensive women were taken as controls between the study period September 2013 to August 2015. Assessment of homocysteine level was done in cases and controls. Association was studied between maternal homocysteine level and preeclampsia and eclampsia and also with the severity of pre-eclampsia.

Results: There was a significant association between pre-eclampsia and eclampsia and maternal serum homocysteine levels (median homocysteine level 16.25µmol/lit with P-value being less than 0.001 for preeclampsia ; median homocysteine level-31.34 µmol/lit with p value being less than 0.001 for eclampsia). There was also a significant association between severity of preeclampsia and maternal homocysteine level (mean serum homocysteine level in nonsevere preeclampsia 14.99±3.47 µmol/lit with p value being 0.35; in severe preeclampsia 19.90±6.17 µmol/lit with p value less than 0.001 and 30.44±4.75 µmol/lit with p value less than 0.001).

Conclusions: In the present study a positive association was found between pre-eclampsia and maternal serum homocysteine levels and it was found to be statistically significant. Also the levels were higher as the severity of preeclampsia increased.

Keywords: Eclampsia, Pre-eclampsia, Maternal serum homocysteine level

INTRODUCTION

Preeclampsia is a pregnancy related hypertensive disorder occurring usually after 20 weeks of gestation. Preeclampsia has remained a significant public health threat in both developed and developing countries contributing to maternal and perinatal morbidity and mortality globally.^{1,2} The problem is compounded by the continued mystery and unpredictable nature of the disease. According to National Health Survey 1998 preeclampsia was the most common medical disorder complicating pregnancy. Incidence of preeclampsia is 5 - 7% of all pregnancies.³ Though the exact cause of

preeclampsia is undecided; endothelial dysfunction with associated intense vasospasm has been implicated in its causation. Recently homocysteine a metabolite of essential amino acid methionine has been postulated to produce oxidative stress and endothelial dysfunction and thereby producing preeclampsia.⁴ The association of hyperhomocysteinemia and preeclampsia has been suggested initially by Decker et al and has not been confirmed by many authors although majority of evidence suggest a positive association.

Homocysteine is a sulfur containing amino acid primarily derived from demethylation of dietary methionine, which

is abundant in proteins of animal origin. It is an essential amino acid required for the growth of cells and tissues in the human body. Elevated circulating homocysteine is a risk factor of endothelial dysfunction and vascular disease such as atherosclerosis and occlusive disorders.⁵ The vascular effects of hyperhomocysteinemia have been proposed to include endothelial cell injury and thrombus formation.⁵ Levels of maternal serum homocysteine normally decreases with gestation, either due to a physiological response to the pregnancy, increase in estrogen, hemodilution from increased plasma volume or increased demand for methionine by both the mother and fetus^{6,7,8}. Maternal hyperhomocysteinemia has been associated with a number of placenta-mediated diseases such as preeclampsia. Homocysteine may prove to be the missing link in etiology of preeclampsia. Further homocysteine levels are known to increase with increasing severity of preeclampsia. The homocysteine-mediated vascular changes are similar to those associated with preeclampsia; therefore, a hypothesis has been proposed that hyperhomocysteinemia may be associated with this condition. Several studies have indicated that homocysteine concentrations are increased in women with preeclampsia (Zeeman et al) but so far there are very few reports concerning hyperhomocysteinemia in patients with eclampsia.

Importantly vitamin status, primarily of folate, vitamin B12 and B6, is a major determinant of hyperhomocysteinemia and thus aptly said "adequate intake of Folic acid and other vitamins by the pregnant woman is the future for prevention of dreadful disease like preeclampsia".

We therefore aimed this study to investigate plasma levels of homocysteine in women with preeclampsia and eclampsia, and to assess whether there is an association between hyperhomocysteinemia and the severity of preeclampsia. Estimation of homocysteine may help to predict and prevent preeclampsia and eclampsia, thus reducing the undesired outcome of pregnancy.

METHODS

A case control study was carried out at Tertiary care hospital during September 2013 to August 2015.

Study population: 120 women were recruited in the study in which 40 cases of preeclampsia, 40 cases of eclampsia and 40 were taken as controls.

Inclusion criteria: Cases: Pregnant women above the gestational age of 20 weeks which were further divided into two groups; women with preeclampsia, with eclampsia Controls: Normotensive women were included in study.

Exclusion criteria: Chronic hypertension, multiple gestation, women on antifolate drugs, abruptio placentae, chronic kidney and cardiovascular diseases

Case: Case was defined on the basis of inclusion and exclusion criteria i.e. diagnosed cases of pre-eclampsia characterized by elevation of blood pressure of more than 140mmHg systolic or more than 90mm of Hg diastolic with proteinuria (more than 300 mg/l in 24 hours specimen/1+by dipstick or greater in random urine sample) after 20 weeks of gestation in previously normotensive non proteinuria pregnant women and preeclampsia complicated by convulsions (eclampsia).

Control: The control constituted of equal number of matched age, parity, socio-demographic status, gestational age; healthy normotensive pregnant women visiting the labour ward.

This study was approved by Institutional Ethical Committee. Written informed consent was obtained from all participants recruited in study after they had been made aware of purpose of study. Particulars of the women were noted such as name, age, symptoms, menstrual history for menarche, last menstrual period and past menstrual cycles, history of present pregnancy. Past obstetric history was asked for duration of marriage, infertility, gravidity and parity status, recurrent abortions, pre-eclampsia, growth restriction, low birth weight, preterm delivery, prematurity, late pregnancy losses, neonatal deaths, and mental retardation in previous pregnancy. Past medical history was asked for any associated medical disorders like diabetes, thyroid disorders, exposure to radiation or autoimmune disorders, epilepsy, consumption of antifolate drug and history of consumption of iron and folic acid during pregnancy. Significant surgical history, family history was also asked. A thorough clinical examination including height, weight, pulse, blood pressure, pedal edema, thyroid enlargement, etc. was done followed by systemic examination. In obstetrical examination gestational age, presentation and amount of liquor was noted and fetal heart sounds were auscultated with stethoscope.

All preliminary and baseline investigations like complete blood count, blood grouping and typing, urine routine and microscopy and blood sugar were done. Ultrasonography was done for fetal growth, liquor and placenta. All investigations pertaining to complications of preeclampsia like liver and kidney function tests, serum uric acid were also done.

Assessment of serum homocysteine levels of cases and controls were done for which 5ml venous blood sample was taken from the antecubital vein irrespective of fasting status (i) In cases, after the diagnosis of pre-eclampsia or eclampsia was made immediate after admission and before the initiation of the antihypertensive treatment and before the delivery and (ii) In controls, after admission. 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 transfused in a plastic vial and stored in refrigeration until analysis. Samples were stored at 2-8°C

(stable upto 14 days when refrigerated). Chemiluminescence immunoassay was used for determining total homocysteine levels in the blood. The system used was an automated, random access, direct CLIA analyzer. This diagnostic test was designed to quantitatively measure Hcy in serum or EDTA plasma. Women were also classified according to the severity of pre-eclampsia into severe and non-severe.

Normal range: 3.7 - 13.9 µmol/lit.

Level more than 13.9 µmol/lit were defined as hyperhomocysteinemia.

Statistical analysis-Statistical comparison between cases and controls was done using Pearson's chi square test, Kruskal Wallis test, odd's ratio, Mann Whitney's test which ever was appropriate, p value below 0.05 was considered to be significant.

RESULTS

Table 1: Distribution of cases and controls according to age, parity and gestational age.

Characteristics	Preeclampsia	Eclampsia	Controls
Age in years (median)*	22	22	23
Nulliparas (%)	50	47.5	67.5
Gestational age in weeks (median)*	37.15	35.35	35.35

*As the data was not evenly distributed median value is calculated.

The case and control group were comparable in age, parity and gestational age in weeks.

Table 2: Distribution according serum homocysteine level in preeclampsia.

Outcome	Homocysteine (µmol/L)			P value
	Median	25 th percentile	75 th percentile	
Preeclampsia Cases (n=40)	16.25	13.82	21.00	<0.001 (VHS)
Controls (n=40)	12.68	10.00	14.15	

Mann Whitney test; p<0.001

Table 3: Distribution according to serum homocysteine level eclampsia.

Outcome	Homocysteine (µmol/L)			P value
	Median	25 th percentile	75 th percentile	
Preeclampsia Cases (n=40)	16.25	13.82	21.00	<0.001 (VHS)
Controls (n=40)	12.68	10.00	14.15	

Mann Whitney test; p<0.001

Median serum homocysteine level for preeclampsia study group was 16.25 µmo;/lit; 25th percentile was 13.82 whereas 75th percentile was 21 µmol/lit. Median serum homocysteine level for control group was 12.68 µmol/lit and 25th and 75th percentile were 10 and 14.15 µmol/lit respectively. It was found that there was significant difference between preeclampsia group and control group with p value of <0.01 (Mann Whitney test) (Table 2).

Median serum homocysteine levels in eclampsia study group was 31.34 µmol/lit; 25th percentile was 28.40 and 75th percentile was 34.56 µmol/lit. Median serum homocysteine level for control group was 12.68 µmol/lit and 25th and 75th percentile 10 and 14.15 µmol/lit. It was found that there was highly significant difference between eclampsia group and control with p value of <0.01 (Mann Whitney test) (Table 3).

Table 4: Association of homocysteine levels with nonsevere preeclampsia, severe preeclampsia and eclampsia.

Group	Mean	p-value
Nonsevere preeclampsia (n=16)	14.99±3.47	0.35
Severe preeclampsia (n=24)	19.90±6.17	<0.001 (VHS)
Eclampsia (n=40)	30.44±4.75	<0.001 (VHS)
Control (n=40)	12.48±2.95	

Bonferroni's test; p<0.001

It is seen that there is significant difference between severe preeclampsia and controls (p<0.001); eclampsia and controls (p<0.001) but there is no statistically difference between nonsevere preeclampsia and control group (p=0.35) (Table 4).

DISCUSSION

Pre-eclampsia is a serious complication of pregnancy with unknown etiology that may occur at any stage of second or third trimester. Pre-eclampsia is a leading cause of maternal and fetal morbidity. Although, the exact cause of pre-eclampsia is still unknown, it is known that in pre-eclampsia the basic pathology is endothelial dysfunction and intense vasospasm. Recently homocysteine, a metabolite of essential amino acid methionine, has been postulated to produce oxidative stress and endothelial cell dysfunction. Elevated plasma homocysteine concentration is an independent risk factor for peripheral vascular diseases and for coronary artery diseases. Serum homocysteine may prove to be the missing link in the etiology of preeclampsia.

Laskowaka M et al and Ingec M et al observed that mean serum homocysteine level for preeclampsia and eclampsia was 10.38±3.5 µmol/lit and 16.7±10.1 µmol/lit respectively.^{9,10} In the present study, median maternal serum homocysteine levels for preeclampsia group,

eclampsia group and control group were 16.25 $\mu\text{mol/lit}$, 31.34 $\mu\text{mol/lit}$ and 12.68 $\mu\text{mol/lit}$ respectively.

Ezzatalsadat Haji Seid Javadi et al reported that the mean maternal serum homocysteine levels in the third trimester of pregnancy was found to be decreased compared to non-pregnant people and serum homocysteine levels in patients with severe preeclampsia were significantly higher ($P < 0.001$) but no significant difference in the mean serum homocysteine level between women with mild preeclampsia and nonpregnant women.¹¹ They emphasized that the low concentration of homocysteine makes trivial changes in vascular endothelium. Khosrowbeygi A, Ahmadvand H revealed that total maternal serum homocysteine level was increased in preeclamptic women and hyperhomocysteinemia was associated with severity of preeclampsia.¹² Acilims YG et al showed that maternal and fetal serum homocysteine levels were found to be significantly higher in severe preeclampsia group than in mild preeclampsia and control group suggesting that elevated level of serum homocysteine might be associated with severity of preeclampsia.¹³ Dudani et al observed that a significant difference in homocysteine level between hypertensive pregnant women and those with mild preeclampsia.¹⁴ In different Studies, a contradictory results have been obtained about the significant increase in homocysteine in mild preeclampsia. The reason for this could be the low concentration of homocysteine slightly affects the endothelium and has a subtle role in occurrence of preeclampsia.

CONCLUSION

This study revealed a direct relationship between serum homocysteine concentrations with severe preeclampsia, eclampsia group and failed to demonstrate the same association with nonsevere preeclampsia group. This study recommends that a multicentric cohort study with large population is needed to support the hypothesis that hyperhomocysteinemia is associated with preeclampsia and its severity. However with regards to results of present study "measurement of homocysteine" can be suggested as a prediction of preeclampsia. To enjoy the healthy period throughout the pregnancy and enjoy the safe motherhood is right of every women.

As the preeclampsia is the commonly encountered and dreadful disease of pregnancy it is important to prevent and diagnose the disease at the earliest to prevent complications and adverse outcomes of pregnancy due to this threat.

This study recommends that a multicentric study with large population is needed to support the hypothesis that hyperhomocysteinemia are associated with preeclampsia and its severity. However with regards to results of present study "measurement of Homocysteine" can be suggested as a prediction of preeclampsia.

By using the serum homocysteine level as predictive tool, we can diagnose the preeclampsia and its severity so we can intervene at the earliest.

Adequate supplementation of Folic acid, B12, and B6 in preconceptional period will help to prevent hyperhomocysteinemia and thus indirectly preeclampsia.

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