Maternal Plasma Homocysteine Level, 24-Hour Urinary Protein and Haemoglobin in Pre-eclamptic Patients: is there Any Relationship?

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Background: Anemia is very common in developing countries especially during pregnancy. Hyperhomocysteinemia can result from genetic or nutrient-related disturbances in the transsulfuration or remethylation pathway for homocysteine metabolism. Inadequate intake of vitamin B_{12}, B_6 or folate may underlie some cases of elevated homocysteine levels. The aim of this study was to investigate the possible relationship between plasma homocysteine level, haemoglobin level and 24-hour urinary protein in pre-eclamptic patients. Folic acid deficiency is one of the major cause of hyperhomocysteinemia which is one of the major risk factor for pre-eclampsia (PET). Severe proteinuria of several grams/day occurs in pre-eclamptic toxaemia.

Methods: A cross-sectional comparative study was carried out on 90 primigravidae attending the "antenatal clinic" of Services Hospital, Lahore. Out of these 60 primigravidae 30 were mildly pre-eclamptic and 30 were severely pre-eclamptic at 30-38 weeks of pregnancy.30 primigravida [30-38 weeks of pregnancy] having uncomplicated pregnancy were taken as control.

Results: The results show that in mild PET and in severe PET, the plasma homocysteine level shows a significant relationship with 24-hour urinary protein and hemoglobin level.

Conclusion: Anemia [folic acid deficiency] is one of the important risk factor in the causation of hyperhomocysteinemia which is one of the major chronic risk factors for eclampsia.

Key-Words: Hyperhomocysteinemia, anemia, mild PET, severe PET.

Introduction

Anemias of diminished erythropoiesis are caused by an inadequate supply of some substances to the bone marrow which are necessary for erythropoiesis.

The most common deficiencies are those of iron, folic acid and vitamin B_{12}.It is estimated that 10% of the population in developed countries and as much as 25-50% in developing countries are anemic. Iron deficiency accounts for most of this prevalence. Increased demands not met by normal dietary intake occur around the world during pregnancy and infancy. When iron deficiency develops there is a decrease in circulating iron, with a low level of serum iron and a rise in serum transferrin iron binding capacity. Ultimately, the inadequacy makes its impact on hemoglobin, myoglobin and other iron compounds.

There are two principal types of megaloblastic anemia; one caused by a folate deficiency and another caused by lack of vitamin B_{12}.These anemias may be caused by a nutritional deficiency [folic acid] or may result from impaired absorption [vitamin B_{12}]. Both of these vitamins are required for DNA synthesis and hence the effect of their deficiency on erythropoiesis are quite similar. High risk of clinically significant folate deficiency associated with poor diet and increased metabolic needs [as in pregnant woman and patients with chronic haemolytic anemias].Inadequate levels of vitamin B_{12} or cobalamin in the body results in pernicious anemia. The metabolic defects induced by vitamin B_{12} deficiency are intertwined with folate metabolism. Vitamin B_{12} is required for recycling of tetrahydrofolate and hence its deficiency reduces availability of the form of folate that is required for DNA synthesis. Both folate and vitamin B_{12} deficiency gives rise to megaloblastic anaemia.[1] So iron deficiency, folic acid deficiency and vitamin B_{12} deficiency lead to anemia and all these deficiencies are common in developing countries especially in pregnancy when metabolic demand is increased.

Homocysteine, a sulfur containing amino acid is an intermediate product of methionine metabolism. It is metabolized through the pathways of transsulfuration and transmethylation.[2] In transmethylation pathway methionine can be regenerated by the transfer of methyl group to homocysteine from N 5-methyltetrahydrofolate, a reaction catalyzed by homocysteine methyltransferase [methionine synthase]. The co-enzyme that mediates this transfer of a methyl group is methylcobalamine derived from vitamin B_{12}. In transsulfuration pathway, homocysteine is an intermediate in the synthesis of cysteine.[3] Hyperhomocysteinemia means increased concentration of homocysteine and it indicates that homocysteine metabolism is compromised causing the export mechanism to remove excess of homocysteine in tissue to blood.[4] Inadequate intake of vitamin B_{12}, B_6 or folate may underly some cases of elevated homocysteine levels.[5]

Hyperhomocysteinemia is associated with cardiovascular and cerebrovascular diseases as well as recurrent miscarriages, placental abruption, pre-eclampsia, intrauterine growth restriction and perinatal death[6].

Pre-eclampsia is pregnancy induced hypertension which includes a triad of clinical signs and symptoms-hypertension, proteinuria and pathologic edema.[7] It is demonstrated that elevated levels of maternal plasma homocysteine are present in pre-eclampsia. Elevated maternal plasma homocysteine plays a role in the pathogenesis of vascular disease in the uteroplacental circulation in placental insufficiency. This role may be locally limited to the placenta when only fetal manifestations are present .In severe pre-eclampsia the role may be extended throughout the maternal vascular tree[5].

The aim of this study was to investigate the possible relationship between plasma homocysteine level, hemoglobin level and 24-hour urinary protein excretion in mildly pre-eclamptic primigravida and severely pre-eclamptic primigravida.

Material and Methods

Following approval by the Local Ethical Committee and patients informed written consent sixty pre-eclamptic patients were included in the study. This cross-sectional comparative study was carried out at Services Hospital, Lahore. Patients were recruited from those attending the "antenatal clinic" and admitted in the "antenatal ward and labor room" of the Services Hospital.

Out of the sixty pre-eclamptic patients thirty patients were of mild pre-eclampsia and thirty patients were suffering from severe pre-eclampsia. All the patients were primigravida and were analyzed in the third trimester of pregnancy (30-38 weeks of pregnancy). All the patients were taking vitamin supplementation irregularly with no history of essential hypertension, diabetes mellitus and Jaundice. An initial interview by a specialist (in gynaecology department) determined the subjects suitability for the trial. Inclusion and exclusion criteria were applied at the interview.

A recent blood sample and urine sample were sent to the laboratory for urine analysis, blood sugar level, serum creatinine level and liver function test to exclude renal disease, diabetes mellitus and liver disease. Twenty-four hours urine was collected according to the standard instructions to the subjects. At the end of each 24-hour collection period, the subjects were asked to empty their bladder completely. 24 hour urinary protein estimation was done by Randox Kit Method.

Inclusion Criteria

For mild pre-eclampsia.

1. Primigravida (30-38 weeks of pregnancy)
2. A diastolic blood pressure 90-100mm Hg and a systolic blood pressure at or above 140mm Hg on at least 2 occasions 6 hours apart.
3. Significant proteinuria more than 300 mg/24 hour.

For severe pre-eclampsia.

1. Primigravida (30-38 weeks of pregnancy).
2. A diastolic blood pressure more than 110mm of Hg on at least 2 occasions 6 hours apart.
3. Significant proteinuria of 4 gram/24 hour or more with any signs and symptoms of impending eclampsia.

Exclusion Criteria

1. Essential hypertension.
2. Renal diseases.
3. Diabetes mellitus.
4. Jaundice.

Each patient was given a full explanation of the study and after taking informed consent a 5ml venous blood was obtained from antecubical vein of patient into vacutainer tubes containing tripotassium EDTA (for preparation of plasma) after an overnight fast. The plasma was removed within an hour and stored at -20^{o}C until analyzed for homocysteine. Plasma total homocysteine level was estimated by a Bio-Rad enzyme linked immunoassay (EIA), microtiter method.

Statistical Analysis

All mean values were expressed as mean + standard deviation (SD). Values of various groups were compared using analysis of variance [ANOVA] . Students "t" test was used to compare means with two categories of study variable. Statistical analysis was carried out using the SPSS® (Statistical Package for Social Sciences), software version 10 for Windows®. P value less than 0.05 was considered significant.

Results

The mean homocysteine level in control group was 5.66+ 0.51μmol/l, in mild PET was 9.67 + 2.83μmol/l and in severe PET it was 9.50+ 1.93 μmol/l. The mean 24-hour urinary protein in control group was 100.03 + 29.91 mg. in mild PET it was 488.97 + 184.59 mg and in severe PET it was 4430.00 + 488.59 mg. The mean plasma homocysteine level and mean 24-hour urinary protein in mild PET and severe PET patients were significantly raised [$p < 0.01$] when compared with their control group.

The mean haemoglobin level in control group was 10.51+ 0.90 gm/dl, in mild PET it was 9.88+ 1.14 gm/dl and in severe PET group it was 9.39+ 0.74 gm/dl. Haemoglobin level also showed a significant [$p<0.01$] decrease in mild PET and severe PET group when compared with their control group [Table 1].

Table 2 shows plasma homo-cysteine level in comparison with mean 24-hour urinary protein and mean haemoglobin levels also show a significant [$p<0.01$] relationship with each other.

Discussion

The plasma homocysteine level in all the three groups in this study can be compared by a study done by Wang and his workers (2000) in which circulating homocysteine levels

in pre-eclampsia were found to be high. The plasma homocysteine level in the control group was 5.9 µmol/l but in the pre-eclamptic patients it was upto 9.4 µmol/L[5]. Similar high levels of about 9.8 + 3.3 µmol/l were found in a study done by Cotter at al (2001)[8]. While in a study done by Lachmeijer et al (2001). it was found that when homocysteine, folate and vitamin B_{12} levels were measured no significant differences in levels were seen between the pooled pre-eclampsia and eclampsia subgroup[9]. In normal subjects 24-hour urinary protein excretion according to McMurray is upto 150 mg/day. While the mean value of 24 hour urinary protein excretion in patients of severe PET was 4.4 ± 0.48 gm.[10] Similar high values have been found in a study done by Mark et al (2002) who showed a value of 4.2 ± 3.6 gm.[11] In this study the mean plasma hemoglobin level show an inverse relationship with plasma homocysteine level. In a study done on Dutch women showed that women with pre-eclampsia with hyperhomocysteinemia had significantly lower folate levels than did women with pre-eclampsia without hyperhomocysteinemia. Vitamin B_{12} levels showed the same significant negative trend. In another study to find out plasma folic acid cutoff value derived from its relationship with homocysteine there is compelling although circumstantial, evidence that low folic acid and high homocyteine are associated with atherosclerosis risk.[12] The aim of this study was to investigate the homocysteine level haemoglobin level and 24-hour urinary protein excretion in mild PET patients and severe PET patients.

Table 1: *Homocysteine level, 24 hour urinary protein and heamoglobin levels in control, mild and severe pre-eclampsia groups*

Study Group	Homocysteine (µMOL/L) Mean ± SD	24 Hour Urinary Protein (MG) Mean ± SD	Haemoglobin Levels (GM/DL) Mean ± SD
Control group (n = 30)	5.66 ± 0.51	100.03 ± 29.91	10.51 ± 0.90
Mild pre-eclampsia (n = 30)	9.67 ± 2.83	488.97 ± 184.59	9.88 ± 1.14
Severe Pre-eclampsia (n = 30)	9.50 ± 1.93	4430.00 ± 488.59	9.39 ± 0.74
***p-value**	p < 0.01 (HS)	P < 0.01 (HS)	P < 0.01 (HS)

* one-way ANOVA was used to test differences between control, mild PET and severe PET groups
" n " stands for number of subjects.

Table 2: *Plasma homocysteine level in comparison with mean 24-hour urinary protein and mean haemoglobin level.*

	Plasma Homocysteine		*p-value
	Upto 7.9 µmol/l	More than 7.9 µmol/l	
24-hour urinary proteins (mg) Mean + S.D	712.12 + 1441.41	2477.0 + 2039.29	< 0.01
Haemoglobin (gm/dl) Mean + S.D	10.34 + 1.05	9.58 + 0.90	<0.01

❖ Student "t" test was used to compare the mean values.

Conclusion

All these studies show that plasma homocysteine level and mean 24-hour urinary protein show a direct relationship with eclampsia because they are increased in eclampsia group as compared to control group. While hemoglobin level show an inverse relationship with eclampsia because hemoglobin level is decreased in eclampsia group as compared to control group. So plasma homocysteine level mean 24-hour urinary protein, hemoglobin level and eclampsia have a significant relationship with each other.

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