ORIGINAL ARTICLE

MATERNAL PLASMA HOMOCYSTEINE LEVEL, 24-HOUR URINARY PROTEIN AND HAEMOGLOBIN IN PRE-ECLAMPTIC PATIENTS: IS THERE ANY RELATIONSHIP?

Sadia Amir, Zamir Ahmed, Adina Shamsi, Kokab Sultana and Qurat-ul-Ain

Background: Anaemia is very common in developing countries especially during pregnancy. Hyperhomocysteinaemia 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 causes 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 primigravida attending the "antenatal clinic" of Services Hospital,Lahore.Out of these 60 primigravida 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 haemoglobin level.

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

Keywords: Hyperhomocysteinemia,anaemia,mild PET, severe PET.

Introduction

Anaemias 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 essential that 10% of the population in developed countries and as much as 25-50% in developing countries are anaemic. 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 haemoglobin, myoglobin and other iron compounds.

There are two principal types of megaloblastic anaemia. One caused by a folate deficiency and another caused by lack of vitamin B_{12}.These anaemias may be caused by a nutritional deficiency [folic acid] or may result from impaired absorption [vitamin B_{12}]. Both of these vitramins 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 anaemias]. Inadequate levels of vitamin B_{12} or cobalamin in the body results in pernicious anaemia. The metabolic defects induced by vitamin B_{12} deficiency are interwined 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 give rise to megaloblastic anaemia.[1] So iron deficiency, folic acid deficiency and vitamin B_{12} deficiency lead to anaemia and all these deficiencies are common in developing countries especally 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] Hyperhomocysteinaemia 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 throughtout the maternal vascular tree.[5]

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

Materials 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 labour room" of the Services Hospital, Lahore between July 2003 to January 2004.

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°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 $\pm$ 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 variables. 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 show a significant [p<0.01] decrease in mild PET and severe PET group when compared with their control group **(Table-1).**

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 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.

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 (m g) 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.*

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 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 haemoglobin 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 hyperhomocysteinaemia. 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.

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 haemoglobin level show an inverse relationship with eclampsia because haemoglobin level is decreased in eclampsia group as compared to control group. So plasma homocysteine level mean 24-hour urinary protein, haemoglobin level and eclampsia have a significant relationship with each other.

Department of Surgery,
Services Hospital/SIMS Lahore.
esculapio@sims.edu.pk
www.sims.edu.pk/esculapio.html

References

1. Robbins. In: Red cell disorders. Robbins. Kumar, Cortan, [eds]. Robbins basic Pathology. 7th ed. W.B Saunders, 2002,Ch 4 ;pp 397- 415
2. Refsum H, Ueland PM, Nygard O, Vollest SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998; 49:31-62.
3. Berg JM, Tymoczko JL, Stryer L, [eds]. Biochemsitry. 5th ed. New York, W.H. Freeman and Company, 2002, ch 24:pp 674-78.
4. Dennis VW, Robbinson K. Homocysteinemia and vascular disease in end-stage renal disease. Kidney International 1996; 50 (S-57): S-11-S-17.
5. Wang J, Trudinger BJ, Duarte N, Wilcken DE, Wand.i-xing.Elevated circulating homoysteine levels in placental vascular disease and associated pre-eclampsia. Br J Obstet Gynaecol 2000; 107:935-38.
6. Steegers-Theunissen RPM, Smith SC, Guilbert LJ, Streegers EAP, Baker PN. Folate affects apoptosis in human trophoblastic cells. Br J Obstet Gynaecol 2000; 107: 1513-15.
7. Hogg BB,Tamura T, Johnston KE,Dubard MB, Goldenberg RL. Second-trimester plasma homocysteine levels and pregnancy-induced hypertension, preeclampsia, and intrauterine growth restriction. Am J Obstet Gynaecol 2000;183:805-09.
8. Cotter M,Molly AM, Scott JM, Daly SF. Elevated plasma homocysteine in early pregnancy. A risk factor for the development of severe pre-eclampsia. Am J obstet Gynecol 2001; 185:781-5.
9. Lachmeijer AM, Arngrimsson R, Bastiaans E, Pals G, Kate LP,de Vries J, et al. Mutations in the gene for methylenetetrahydrofolate reductase, homocysteine levels, and vitamin status in women with a history of pre-eclampsia. Am J obstet Gynecol 2001;184:394-402
10. McMurray JR. In: Proteins in urine, Cerebrospinal fluid and other fluids. Gowenlock AH, McMurray JR, Mclauchlan DM, [eds]. Varleys Practical Clinical Biochemsitry. 6th ed. Oxford, Heinemann, 1998, ch 20: 436-52.
11. Mark C, Jeffrey CL, Ivester TS, Barton JR, Sibai BM. Late postpartum eclampsia: A preventable disease? Am J Obstet Gynaecol 2002;186: 1174-7.
12. Brouwer J, Werten E, Reijngoud D, Jasper J Doarmaal V, Muskiet F. Plasma folic acid cutoff with homocysteine. J clin Chem 1998; 44 (7) : 1545-.550