Research Article

Maternal &umbilical cord plasma homocysteine concentrations in pre-eclamptic pregnancy

Miami A. Ali, FICOG.
Department of Obstetrics& Gynecology, College of medicine.AL- Mustansiriya University, Baghdad, Iraq.

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Address for Correspondence:
Dr. Miami A. Ali, FICOG
Department of Obstetrics &Gynecology, College of medicine.AL- Mustansiriya University PO Box 14132,Baghdad,Iraq.
Email
dr_miami123@yahoo.co.uk

Abstract

Back ground: Serum concentrations of homocysteine decrease during normotensive pregnancy, but increases in pre-eclampsia like some other pregnancy complications. Hyperhomocysteinemia has been hypothesized to be associated with placental micro vascularization disease.
Objectives: To evaluate the concentration of maternal &umbilical homocysteine in pre-eclamptic women in comparison with that of normotensive pregnant women.
Study design: A case control study.
Setting: Department of Gynecology and Obstetrics at Al-Yarmouk Teaching Hospital for a period of one year from April 2010 to April 2011.
Patients & methods: Hundred singleton pregnant women with gestational age between 37 to 40 weeks were divided into two groups: (Study group): 50 pregnant patients with pre-eclampsia , 30 of them presented with severe pre-eclampsia and 20 presented with mild pre-eclampsia were compared to (control group) which include 50 normotensive pregnant patients. Bloods was collected from women in both groups, serum homocysteine and cord blood homocysteine were measured in patient with pre-eclampsia and were compared with that of normotensive patient.
Results: Mean maternal serum homocysteine level in comparison between the study & the control groups was highly significant as the P value was (0.0001).Mean umbilical homocysteine level in comparison between the study & the control groups was highly significant as P value was (0.0001).Maternal homocysteine levels were found to have a significant strong direct correlation with the umbilical homocysteine in the severe pre-eclampsia since the r value was (0.808) while in the mild and the control groups, they were moderately correlated as the r value was (0.536,0.526) respectively.
Conclusion : This study has shown a significant increase in homocystein concentration in maternal and umbilical cord blood of the fetuses in pre-eclamptic women compared with the values obtained from women of normal pregnancy.

Keywords: pre-eclampsia, maternal homocysteine ,cord blood homocysteine

INTRODUCTION

Pre-eclampsia (PE): is a syndrome unique to pregnancy characterized by the new onset of hypertension and proteinuria after 20 weeks of gestation in a woman whose blood pressure was previously normal . PE is called the" disease of theories," because genetic, immunologic, vascular, hormonal, nutritional, and behavioral factors have all been proposed as causes [(1)]. Oxidative stress is the imbalance of pro-oxidants (homocysteine, low density lipoprotein, hypertriglyceridemia, increased iron), and antioxidants (high-density lipoprotein and transferrin, a blood protein that binds with iron) leading to the formation of oxygen free radicals and lipid peroxides, lipid peroxides and oxygen free radicals are directly toxic to the endothelial cells and increase the release of cytokines, which cause damage to the endothelial lining of blood vessel walls [(2)]. Increase in plasma concentration of homocysteine (Hcy) is common in patients with stroke, peripheral vascular disease [(3)], and coronary disease [(4)], and confer an independent risk of atherosclerosis [(5)]. Normal human plasma contains total homocysteine concentrations below 16 micromole/L [(6)]. Much of the endothelial

dysfunction attributed to homocysteine is thought to occur primarily from oxidative stress [7]. Studies have shown that homocysteine suppresses the vasodilator nitric oxide [8,9] . It has been shown that alterations in the methionine-homocysteine metabolism (MHM) may be related to the systemic damage that leads to the classical clinical picture of PE [10]. In fact, in normal pregnant women, plasma concentrations of homocysteine are low in the first trimester of pregnancy and reach their lowest level in the second half of gestation [11] , whereas hyperhomocysteinemia has been described in women who would develop PE [12] .The aim of this study was to evaluate the concentration of maternal & umbilical homocysteine in pre-eclamptic women in comparison with that of normotensive pregnant women.

PATIENTS AND METHODS

A case control study was conducted at the Department of Obstetrics &Gynecology at Al-Yarmouk Teaching Hospital for a period of one year from (April 2010 to April 2011) . The study was approved by the local Medical Research Ethics Committee of College of Medicine , Almustansiryia University, Department of Obstetrics & Gynecology. Verbal consent was obtained from all participants before enrolling in the study. The study included 100 pregnant women who were divided into study group and control group. The **study group** (S) include fifty PE women who who were admitted to the obstetric ward for evaluation and then were delivered, were further sub divided into severe PE: included 30 pregnant women & 20 pregnant women presented with mild PE. According to the American College of Obstetricians and Gynecologists patient was diagnosed as having severe PE if their blood pressure of ≥160 /110 in addition to proteinuria ≥ +2 or presented with other concurrent parameters: persistent headache, visual disturbances, epigastric pain or maternal or fetal complications, were diagnosed as mild PE if their blood pressure was less than 160/110 with absents of any maternal or fetal complications and the **control group** (C) included 50 singleton normotensive pregnant women, their BP<140/90mmHg & proteinuria was nil who were admitted with signs and symptoms of labour. **Inclusion criteria:** For both the S and the C groups: Singleton pregnancy, delivery between 37 and 4o weeks & birth weight of 2.5 to 4.5 kg. **Exclusion criteria:** Multiple pregnancies**,** women with medical illness like diabetes, infections, and metabolic, nutritional or hemorrhagic complications, fetal congenital malformation, birth asphyxia, abnormal placentation, oligohydramnios or polyhydramnios, substance abuse (alcohol, tobacco abuse, illicit drugs, etc.). The demographic characteristics of each patient were assessed including maternal age, gravidity, parity, past medical, surgical and obstetric history. For both groups the gestational age was calculated from the first day of last menstrual period and confirmed with early ultrasound scan. Complete examination (general and obstetric examination) was done for all patients. The following investigations were done for all patients in the S & the C groups: Mid stream urine examination, full blood count, coagulation profile, liver function test, renal function test, maternal homocystein and abdominal ultrasound. After delivery cord blood samples were obtained for the measurement of homocysteine. 3ml of venous blood was taken from each patient by venipuncture and after delivery; umbilical cord blood was collected by direct venipuncture of the umbilical vein after of delivery. The specimen was centrifuged at 2000RPM for 10 minutes and the plasma removed and either tested immediately or frozen at -70˚C until testing homocysteine.

Statistical analysis: Analysis of data was carried out using the available statistical package of SPSS-18 (Statistical Packages for Social Sciences- version 18 "PASW"). Data were presented in simple measures of frequency, percentage, mean, and standard deviation. The significance of difference was tested using analysis of variance (ANOVA) for more than two groups. Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation to determine the relationship between two quantitative variables, r=correlation coefficient .

RESULT

There was no statistically significant difference in the mean maternal age& the parity between the S& the C groups, since the p value were 0.954 &0.963 respectively. There was statistically significant difference in the mean gestational age between the S& the C groups, since the p value (0.0001). There was statistically significant difference in the mean birth weight since the p value was (0.0001) as shown in table 1.

Maternal plasma homocysteine levels were observed to be highest in the severe PE group compared to the mild PE and control groups as the mean value for the severe, mild, and the control was (7.156,5.561,5.448) respectively .since the P value was (0.0001) when compared severe pre-eclampsia to control and P value was (0.0001) when compared severe PE to mild PE, as shown in table 2

Table1: The demographic characteristic and birth weight in both the study & the control groups:

	Severe PE	Mild PE	Controls	P value
Age (years)	29.63±4.63 (20-36)	29.35±3.87 (24-35)	29.40±3.10 (24-35)	0.954
Parity	1.40±1.16 (0-4)	1.50±1.50 (0-4)	1.46±1.31 (0-4)	0.963
Gestational age (weeks)	37.70±0.88 (37-40)	37.80±1.01 (37-40)	38.72±1.05 (37-40)	0.0001*
Birth weight (grams)	**2672.9± 397.75 (2000-3400)**	**3197.5± 172.04 (2900-3500)**	**3495.6± 141.46 (3100-3800)**	**0.0001***

Data were presented as Mean ±SD (Range)

*Significant using ANOVA test for difference among more than two independent means at 0.05 level of significance.

Maternal plasma homocysteine levels were observed to be highest in the severe PE group compared to the mild PE and control groups as the mean value for the severe, mild, and the control was (7.156,5.561,5.448) respectively .since the P value was (0.0001) when compared severe pre-eclampsia to control and P value was (0.0001) when compared severe PE to mild PE, as shown in table 2

Umbilical homocysteine levels were observed to be highest in the fetuses of patients with severe PE compared to the fetuses of patients with mild PE and control groups and the P value compared to mild PE was(0.0001)and compared to control group was(0.0001) as shown in table 3.

According to the Pearson correlation test, maternal homocysteine was found to have a significant strong direct positive correlation with umbilical homocysteine in severe PE as the r value was(0.808)while in the mild and control groups, it was moderately correlated as the r value were (0.536,0.526) respectively as shown in table 4.

Sensitivity and specificity of homocysteine level as predictor of adverse pregnancy outcome:

Depending on previous data, analysis was done to find the sensitivity and specificity of maternal homocysteine level in predicting the outcome of pregnancy in women with PE, results showed that the test has sensitivity of 60 % and specificity of 62% at cut of value of 5.830Mmol/L. And of umbilical homocysteine the sensitivity of 66% and specificity of 90% at cut of value of 5.965 Mmol/L. as shown in figure 1 and table 5.

Table 2:Maternal plasma homocysteine levels in both the study and the control groups:

	Maternal Homocystein (Mmol/L)		
	Severe PE	Mild PE	Control
Number of patient	30	20	50
Mean Hcy (Mmol)	7.156	5.561	5.448
Std. Deviation	1.032	1.151	0.613
P value compared to control	0.0001*		-
P value compared to Mild PE	0.0001*	-	-
***Significant using Student-t-test for difference between two independent means at 0.05 level of significance**			

Table3: Distribution of umbilical homocysteine in both the study & the control groups:

	Umbilical Homocystein (Mmol/L)		
	Severe PE	Mild PE	Control
Number of patient	30	20	50
Mean of umbilical Hcy (Mmol)	8.606	5.536	5.343
P value compared to control	0.0001*	0.276	-
P value compared to Mild PE	0.0001*	-	-
***Significant using Student-t-test for difference between two independent means at 0.05 level of significance**			

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Table 4:The coefficient correlation(r) of maternal & umbilical plasma homocysteine in comparison between the study & the control groups.

		Maternal Homocystein (Mmol/L)		
		Severe PE	Mild PE	Controls
Umbilical Homocystein (Mmol)	R	0.808**	0.536*	0.526*
	P	0.0001	0.015	0.015

***Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level**

The Pearson correlation coefficient (r). * Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

The r<0.5 weak correlation, >0.5 moderate strength, >0.7 strong correlation.

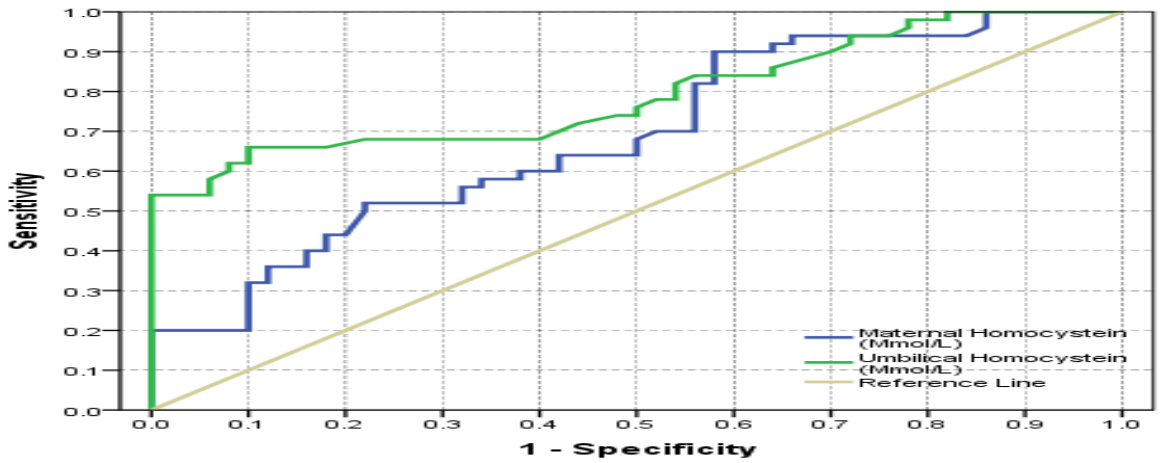

Fig 1: The ROC curve of homocysteine level between pre-eclamptic and control groups:

Table 4 Criterion values and coordinates of the ROC curve

Positive if Greater Than or Equal To	Sensitivity	Specificity
Maternal Homocysteine(Mmol/L):4.230		
	100%	14.0%
4.465	98.0%	14.0%
4.510	96.0%	14.0%
5.465	74.0%	44.0%
5.830	60.0%	62.0%
6.410	52.0%	76.0%
6.430	52.0%	78.0%
6.445	50.0%	78.0%
Umbilical Homocysteine (Mmol/L): 4.600		
	100%	18.0%
4.705	98.0%	18.0%
4.765	98.0%	20.0%
4.810	98.0%	22.0%
5.965	68.0%	78.0%
5.985	66.0%	82.0%
5.995	66.0%	90.0%
6.060	**64.0%**	**90.0%**

DISCUSSION

Hyperhomocysteinemia has been demonstrated to be associated with PE by many authors [13,14], (hcy) might play a role in promoting endothelial dysfunction. Therefore, the goal of this study was to evaluate the possible association between plasma total (hcy) and severity of PE in pregnant women. The current study showed that maternal and fetal plasma levels of (hcy) were elevated in patient with severe PE compared to mild PE and control groups. **In 1969, McCully** [15] recognized that an elevation in the concentration of plasma (hcy) can lead to an increased risk of cardiovascular disease. **Rajkovic et al; 1997** find that the (hcy) mediated vascular changes are similar to those associated with PE therefore hypothesis has been proposed that hyper homocysteinemia may be associated with this condition, and in 1999 evaluated postpartum plasma (hcy) concentration and found that it was higher among women with eclampsia and pre-eclampsia compared with normotensive women [13]. The following investigator**: Walker et al; 1999 [17], Hogg et al; 2000 [18], Vollset et al; 2000 [19]** have demonstrated the relationship between hyperhomocysteinemia and PE. **Wang et al; 2000** [20] noted that cases of PE with the most evidence for umbilical placental vascular disease had no increase in fetal (hcy) levels, leading them to suggest that the placental vascular disease itself impairs the transport of(hcy) . **Drzewoski et al; 2000** [21] reported in their study that elevated blood levels of (hcy) is strongly related to an increased risk for atherosclerosis and cardiovascular disease. In a study done by **Cotter et al; 2001**[22] concluded that in early pregnancy increased (hcy) may be associated with a 4-fold increased risk for development of non severe PE. In a study done by **Gurbuzet al; 2004**[23] found that (hcy) concentration to be raised in PE and the level in eclampsia being higher than that in severe PE.in asstudy done in Japan found that a high total homocysteine (tHcy) level during pregnancy is a risk factor for adverse perinatal outcomes, such as fetal growth restriction and preeclampsia[24]. So serum homocysteine concentration tends to be positively correlated with the degree of severity of disease. However some studies showed no significant difference of (hcy) concentration between pre-eclampsia and eclampsia [25].

So in conclusion this study had shown a significant increased in homocystein concentration in maternal and umbilical cord blood of the fetuses in PE women compared with the values obtained from women of normal pregnancy and this increment was directly correlated with the severity of PE.

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