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Homocysteine and its Association with Lipid Peroxidation and Leptin in Preeclampsia

A. Khosrowbeygi, N. Lorzadeh, H. Ahmadvand and Y. Shiravand
School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

Corresponding Author: Ali Khosrowbeygi, Department of Biochemistry, School of Medicine, Lorestan University of Medical Sciences (Pardis Kamalvand), Box No. 381351698, Khorramabad, Iran

ABSTRACT

The aim of the present study was to determine the relationship of maternal serum levels of total homocysteine with adipocytokine leptin and oxidative stress assessed by levels of 8-Isoprostane and malondialdehyde in preeclampsia. The study population consisted of 30 preeclamptic patients and 30 matched healthy pregnant women. Serum levels of total homocysteine, 8-Isoprostane and total leptin were assessed using commercially available enzyme-linked immunosorbent assay methods. The amount of malondialdehyde was determined by the thiobarbituric acid assay. Statistical analysis was performed using the Mann-Whitney U test and Spearman's correlation analysis. Serum levels of homocysteine, 8-Isoprostane, malondialdehyde and leptin were significantly increased in preeclamptic group compared with normal pregnant women. Levels of total homocysteine correlated positively with both 8-Isoprostane and malondialdehyde in preeclamptic group, while no association was found with adipocytokine leptin. The results of the present study showed that in preeclamptic women, maternal serum levels of total homocysteine were correlated with oxidative stress but not with leptin.

Key words: Homocysteine, isoprostane, malondialdehyde, leptin, pre-eclampsia

INTRODUCTION

Preeclampsia, a pregnancy specific syndrome, is a major cause of maternal and perinatal morbidity and mortality. The exact mechanism underlying etiology of preeclampsia remains elusive (Abbasalizadeh *et al.*, 2007; Ghazavi *et al.*, 2008; Moslemizadeh *et al.*, 2008; Savvidou *et al.*, 2008). It has been proposed that excess oxidative stress and exaggeration of a maternal inflammatory response are involved in the pathogenesis of preeclampsia. Endothelial dysfunction, insulin resistance and inflammation are demonstrated features of preeclampsia which share with atherosclerosis (Shakour-Shahabi *et al.*, 2010; Shenoy *et al.*, 2010).

Homocysteine, a sulfur containing amino acid, is involved in processes such as lipid peroxidation and oxidative stress (Baumann *et al.*, 2007). Hyperhomocysteinemia is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis (Joshaghani *et al.*, 2007). It has been hypothesized that maternal hyperhomocysteinemia to be associated with a number of placenta-mediated diseases such as preeclampsia (Hoque *et al.*, 2008).

Leptin is an adipocytokine that is secreted primarily by white adipose tissue. It is also produced by human placental trophoblasts. Leptin stimulates inflammatory responses via induction of proinflammatory cytokines in placenta and adipose tissue. It is also recognized to have many vascular effects (Iftikhar *et al.*, 2008; Mumtaz *et al.*, 2008).

It has been hypothesized that oxidative stress can cause the releasing of leptin derived from placenta into maternal circulation (Ouyang *et al.*, 2009). On the other hand, it has been proposed that homocysteine promotes oxidative stress that resulting in lipid peroxidation (Coppola *et al.*, 2000; Perna *et al.*, 2003; Splaver *et al.*, 2004; Weiss, 2005; Castelao and Gago-Dominguez, 2008). Therefore, the authors hypothesized that serum levels of homocysteine would be associated with leptin in preeclampsia. Little studies have been done to evaluate simultaneously maternal circulating levels of homocysteine, 8-Isoprostane, malondialdehyde and leptin in preeclampsia. Therefore, the purpose of the present study was to determine whether maternal serum levels of total homocysteine are associated with adipocytokine leptin and oxidative stress assessed by levels of 8-Isoprostane and malondialdehyde in preeclamptic women.

MATERIALS AND METHODS

Subjects: A cross-sectional study was designed. The study was conducted from May 2009 to July 2010. It was approved by Institutional Ethical Review Board and informed consent was obtained from each pregnant woman enrolled in this study. Sixty pregnant women were included in the present study. Of these 30 women were preeclamptic patients and 30 age-, gestational week- and Body Mass Index (BMI) - matched were as normal group. Preeclampsia was defined as blood pressure equal to or higher than 140/90 mm Hg with proteinuria of either higher than 100 mg dL⁻¹ by urine analysis or higher than 300 mg in a 24 h urine collection. Severe preeclampsia was defined as blood pressure equal to or higher than 160/110 mmHg (Khosrowbeygi and Ahmadvand, 2009). Exclusion criteria were smoking, multiple gestation, diabetes mellitus, chronic hypertension, heart failure, renal disease, inflammatory or infective disorders, infectious disease, and treatment with antifolate drugs (Hasanzadeh *et al.*, 2008). All of the samples were being supplemented with vitamins B and folate during pregnancy. Blood samples were collected from preeclamptic patients at the time of acceptance to the hospital shortly after the preeclampsia diagnosis was confirmed. Serum samples were stored at -70°C until examination.

Measurements: Total homocysteine levels were measured by commercially available enzyme immunoassay (EIA) method (Axis Homocysteine EIA, Axis-Shield Diagnostic Ltd, United Kingdom). The procedure for the EIA was according to the instructions provided by the manufacturer. The sample volume that used was 25 µL. Absorbance was measured at a wavelength of 450 nm using enzyme-linked immunosorbent assay (ELISA) reader (STAT FAX 2100, USA) and the concentration of total homocysteine was presented as µmol L⁻¹. The intra-assay coefficient of variation was <10%. The sensitivity of the assay was 2.0 µmol L⁻¹.

In the present study free form of 8-Isoprostane was assessed. At first, free 8-Isoprostane was purified by affinity chromatography method using commercially available affinity column (Cayman Chemical, Ann Arbor, MI, USA) (Khosrowbeygi and Zarghami, 2007). All samples were centrifuged briefly at 1500 rpm for isolating of particulates and precipitates. Then the supernatant was diluted 1:5 with column buffer and applied to the column. Other procedures were according to the instructions provided by the manufacturer. The ethanol washed 8-Isoprostane stored at -20°C until measurement. For measuring 8-Isoprostane, the elution solution was evaporated to dryness under a stream of nitrogen gas. The dried samples were dissolved in buffer. Then, the concentration of free 8-Isoprostane was measured by EIA method. We used commercially available EIA method (Cayman Chemical, Ann Arbor, MI, USA). The procedure for the EIA was according to the instructions provided by the manufacturer. The sample volume that used was 50 µL.

Absorbance was measured at a wavelength of 405 nm using ELISA reader and levels of 8-Isoprostane were presented as pg/mL. The intra-assay coefficient of variation was <10%. The detection limit and specificity of 8-Isoprostane assay were 5 pg mL⁻¹ and 100%, respectively.

The amount of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) assay (Mihara and Uchiyama, 1978). All reagents were obtained from Merck Company (Darmstadt, Germany). Briefly, 1 vol of serum was added to 6 vol of phosphoric acid [1.00% (w/v)] in 0.1 N HCl, 2 vol of TBA [0.60% (w/v)] in 0.1 N HCl. The samples were heated in a boiling water bath for 45 min. After cooling, the chromogen was extracted in 8 vol of n- butanol. The absorbance of the organic phase was measured at a wavelength of 532 nm. The concentration of MDA was expressed as µmol/l using a molar absorption co-efficient of 156000 M⁻¹ cm⁻¹.

The levels of total leptin were measured using commercially available human leptin ELISA method (BioVendor Laboratory Medicine, Inc. Czech Republic). The procedure for the method was according to the instruction provided by the manufacturer. Absorbance was measured at a wavelength of 405 nm using ELISA reader. The levels of leptin were presented as ng mL⁻¹. The intra-assay coefficient of variation of the method was <10%. The sensitivity and specificity of the leptin assay were 0.2 ng mL⁻¹ and 100%, respectively.

Statistical analysis: Based on a literature review, using an α value of 0.05 and a β value of 0.2 (80% power), the minimum sample size required was calculated 30 samples per group. Differences between case and control groups were assessed using Mann-Whitney U test. Coefficients of correlation were calculated using Spearman's correlation analysis. All hypothesis tests were two-tailed with statistical significance assessed at the p value <0.05 level with 95% confidence intervals. The levels of leptin were adjusted for BMI (Abbasi *et al.*, 2006). The data are expressed as the Mean±SEM. Statistical computations were calculated using SPSS 11.5 for windows software (SPSS Inc, Chicago, IL, USA).

RESULTS

Characteristics of normal pregnant women and preeclamptic patients are shown in Table 1. There were no significant differences in age, gestational age and BMI between preeclamptic and normal pregnant women. Systolic and diastolic blood pressures were significantly higher in preeclamptic women than those in healthy pregnant women. Women with preeclampsia and normal pregnant women had systolic blood pressure ranged from 130-180 and 90-110 mm Hg, respectively. Preeclamptic patients and healthy pregnant women had diastolic blood pressure ranged from 80-120 and 60-80 mmHg, respectively.

Maternal serum levels of total homocysteine, 8-Isoprostane, malondialdehyde and leptin in preeclamptic and normal pregnant women are shown in Table 2. Compared with normal group, serum levels of homocysteine, 8-Isoprostane, malondialdehyde and leptin were significantly

Table 1: Characteristics of preeclamptic and normal pregnant women

Parameter	Normal pregnant (n = 30)	Preeclamptic (n = 30)	p-value
Age (years)	31.07±0.35	32.27±0.69	0.20
Gestational age (weeks)	39.10±0.23	38.50±0.24	0.09
Third trimester BMI (kg m ⁻²)	26.23±0.51	27.88±0.78	0.10
Systolic blood pressure (mmHg)	109.00±1.11	150.67±1.91	0.001
Diastolic blood pressure (mmHg)	66.33±1.31	95.67±2.23	0.001

Results are presented as Mean±SEM. BMI, body mass index

Table 2: Maternal serum levels of total homocysteine, 8-Isoprostane, malondialdehyde and leptin in preeclamptic and normal pregnant women

Parameter	Normal pregnant (n = 30)	Preeclamptic (n = 30)	p-value
Homocysteine ($\mu\text{mol L}^{-1}$)	6.38±0.30	14.05±1.43	0.00
8-Isoprostane (pg mL^{-1})	37.06±2.77	202.97±17.39	0.00
Malondialdehyde ($\mu\text{mol L}^{-1}$)	3.81±0.48	5.00±0.29	0.03
Leptin (ng mL^{-1})	19.69±0.28	20.75±0.48	0.04

Results are presented as Mean±SEM

Table 3: Maternal serum levels of total homocysteine, 8-Isoprostane, malondialdehyde and leptin in mild and severe preeclampsia (PE) compared with normal pregnancy

Parameter	Normal pregnancy (n =30)	Mild PE (n = 17)	Severe PE (n = 13)	P ^a	P ^b	P ^c
Homocysteine ($\mu\text{mol L}^{-1}$)	6.38±0.30	11.49±1.19	17.40±2.69	0.00	0.00	0.03
8-Isoprostane (pg mL^{-1})	37.06±2.77	202.58±27.04	203.48±20.3	0.00	0.00	0.98
Malondialdehyde ($\mu\text{mol L}^{-1}$)	3.81±0.48	3.97±0.14	6.36±0.42	0.85	0.00	0.00
Leptin (ng mL^{-1})	19.69±0.28	20.41±0.69	21.20±0.66	0.26	0.02	0.42

Results are presented as Mean±SEM. ^aStatistical analysis of difference between mild PE and normal pregnancy. ^bStatistical analysis of difference between severe PE and normal pregnancy. ^cStatistical analysis of difference between mild and severe PE

increased in preeclamptic group. Preeclamptic and normal pregnant women had total homocysteine levels ranged from 6.30-36.19 and 4.21-12.41 $\mu\text{mol L}^{-1}$, respectively. Preeclamptic and healthy pregnant women had 8-Isoprostane values ranged from 101.53-489.70 and 17.4-85.30 pg mL^{-1} , respectively. Patients and normal women had malondialdehyde levels ranged from 3.23-8.85 and 0.58-7.95 $\mu\text{mol L}^{-1}$, respectively. Preeclamptic and normal pregnant women had leptin values ranged from 17.18-27.08 and 17.29-21.89 ng mL^{-1} , respectively.

Patients were then stratified to mild and severe preeclampsia (Table 3). Women with severe and mild preeclampsia had total homocysteine levels ranged from 6.48-36.19 and 6.30-24.59 $\mu\text{mol L}^{-1}$, respectively. Severe and mild preeclamptic patients had 8-Isoprostane values ranged from 118.18-489.70 and 101.18-320.55 pg mL^{-1} , respectively. Patients with severe and mild preeclampsia had malondialdehyde levels ranged from 4.47-8.85 and 3.23-5.36 $\mu\text{mol L}^{-1}$, respectively. Severe and mild preeclamptic women had leptin values ranged from 18.40-27.08 and 17.17-24.74 ng mL^{-1} , respectively. Women with severe preeclampsia had higher serum levels of total homocysteine and malondialdehyde than mild preeclamptic patients. Serum levels of homocysteine and 8-Isoprostane were significantly higher in mild preeclampsia than those in normal pregnancy. Serum levels of homocysteine, 8-Isoprostane, malondialdehyde and leptin were significantly increased in severe preeclamptic group compared with normal pregnant women.

Levels of total homocysteine correlated positively with both 8-Isoprostane (Fig. 1) and malondialdehyde (Fig. 2) in preeclamptic group. No association was found between serum levels of homocysteine and adipocytokine leptin in women with preeclampsia. Levels of total homocysteine correlated positively with systolic blood pressure values in preeclamptic women ($r = 0.44$, $p = 0.01$).

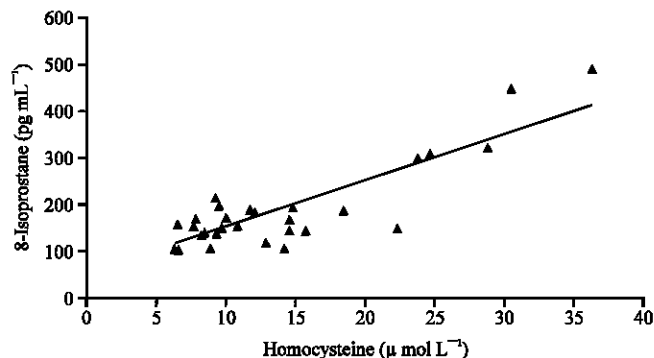


Fig. 1: Correlation between maternal serum levels of total homocysteine and 8-Isoprostane in preeclamptic women ($r = 0.81$, $p = 0.000$)

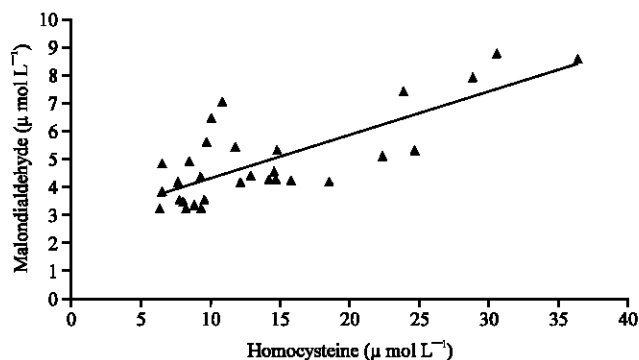


Fig. 2: Correlation between maternal serum levels of total homocysteine and malondialdehyde in preeclamptic women ($r = 0.77$, $p = 0.000$)

DISCUSSION

The most relevant finding of this study is that maternal serum levels of total homocysteine correlated positively with both 8-Isoprostane and malondialdehyde in preeclampsia but not with adipocytokine leptin.

The findings of the present study were in line with the studies reporting the increased maternal serum levels of homocysteine in preeclamptic patients compared with normal pregnant women (Mao *et al.*, 2010; Makedos *et al.*, 2007; Atis *et al.*, 2010). In uncomplicated pregnancies maternal circulating level of homocysteine is decreased, probably resulting from hemodilution, increased glomerular filtration rate, hormonal changes, increased uptake of the amino acid by fetus (Ingec *et al.*, 2005) and increasing in enzymatic activity related to homocysteine metabolism (Dasarathy *et al.*, 2010).

The results of the present study were in line with Harsem *et al.* (2006) study reporting the increased lipid peroxidation assessed as circulating levels of 8-Isoprostane in preeclamptic patients compared with normal pregnant women. However, our data were not agreed with Ishihara *et al.* (2004) study that showed there is no significant difference in 8-Isoprostane levels between preeclampsia and normal pregnancy. In the present study, maternal serum levels of malondialdehyde were significantly increased in preeclamptic patients compared with normal pregnant women. This finding was in concordance with earlier reports (Sharma *et al.*, 2006; Biri *et al.*, 2007; Howlader *et al.*, 2007; Mehendale *et al.*, 2008; Uboh *et al.*, 2008).

The data of the present study confirmed the results obtained by Ouyang *et al.* (2007) and Nakatsukasa *et al.* (2008), who observed that circulating levels of leptin are significantly higher in preeclamptic patients than in normal pregnant women.

Vitamins B6, B12 and folate play central roles in homocysteine metabolism. If B vitamins are not present in adequate amounts to support these metabolic changes, then the natural decrease of homocysteine might not occur and hyperhomocysteinemia can develop. Vitamin B deficiencies might thus confer a greater risk of preeclampsia on the mother. Hyperhomocysteinemia caused by the methylenetetrahydrofolate reductase C677T mutation, can be corrected with folic acid administration (Alshatwi, 2007). There are some conflicting findings about the association of hyperhomocysteinemia and maternal circulating levels of B vitamins in preeclamptic women. D'Anna *et al.* (2004) report suggests that deficiencies in B vitamins and folate are associated with an increased risk of preeclampsia. However, Acilmis *et al.* (2011) study showed that maternal circulating concentrations of vitamins B12 and folic acid are not significantly different in hyperhomocysteinemic preeclamptic group compared with normal pregnant women. Lopez-Quesada *et al.* (2003) found that the levels of homocysteine and folate are increased in preeclampsia but values of vitamin B12 are not changed compared with normal pregnancy. In the present study, all of the samples were being supplemented with vitamins B and folate during pregnancy. Therefore, the hyperhomocysteinemia observed in the preeclamptic women might not be due to the vitamins deficiency.

One mechanism by which hyperhomocysteinemia has been proposed to influence its pathological effects is by promoting increased oxidative stress that resulting in lipid peroxidation at the cell surface (Ravari *et al.*, 2009). An association of elevated plasma homocysteine levels with enhanced *in-vivo* lipid and protein oxidation in humans is suggested by the correlation of plasma homocysteine with plasma 8-Isoprostanes in hyperhomocysteinemic men (Voutilainen *et al.*, 1999).

Preeclampsia seems to originate from complex interactions among maternal constitutional factors, including pre-existing metabolic abnormalities, placenta-derived products and the exaggerated adaptive mechanisms that normally occur during pregnancy which are strikingly similar to abnormalities associated with cardiovascular disease. In addition, similar to cardiovascular diseases, endothelial dysfunction plays a critical role in the pathogenesis of preeclampsia. The interplay among endothelial dysfunction and ongoing oxidative stress, inflammation and the hypercoagulable state that are present in preeclampsia appears quite complex as these mechanisms may potentiate each other, resulting in cumulative vascular damage. Hyperhomocysteinemia is a risk factor for endothelial dysfunction and vascular disease such as atherosclerosis. Leptin is a marker of increased risk for cardiovascular disease. Elevated levels of leptin are suggestive of resistance to its metabolic effects and may promote platelet aggregation, thus further contributing to a hypercoagulable state of preeclampsia. Oxidative stress due to free radical generation also contributes to endothelial dysfunction both in preeclampsia and atherosclerosis (Craici *et al.*, 2008). It has been shown that there is no significant correlation between plasma levels of leptin and homocysteine in coronary artery disease (Sainani and Karatela, 2009).

The results of the present study were in agreement with a previous report by Tug *et al.* (2003), who observed that circulating levels of homocysteine are correlated positively with malondialdehyde in preeclamptic patients. The present study also showed a significant positive correlation between maternal serum levels of homocysteine and 8-Isoprostane. Measurement of 8-Isoprostane may provide a reliable marker of lipid peroxidation *in vivo*, because, it is a stable compound. In addition, 8-Isoprostane is specific product of free radical-induced lipid peroxidation (Pratico *et al.*, 2001).

CONCLUSION

In summary, maternal serum levels of total homocysteine were correlated with oxidative stress but not with adipocytokine leptin in preeclampsia. This finding might support the proposed mechanism that hyperhomocysteinemia contributes to the pathogenesis of preeclampsia by inducing general oxidative stress that results in lipid peroxidation at vascular cells.

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