

Endothelial Dysfunction in Preeclampsia

Increased Homocysteine and Decreased Nitric Oxide Levels

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Key Words

Preeclampsia · Endothelial dysfunction · Homocysteine · Nitric oxide

Abstract

Endothelial dysfunction underlies the pathogenesis of preeclampsia, but its mechanism has not yet been completely understood. Elevated oxygen free radicals may partially explain the endothelial cell damage. In this study, we have aimed to measure homocysteine (Hcy) and nitric oxide (NO) levels as endothelial dysfunction markers in preeclamptic women. Nineteen preeclamptic (33.9 ± 1.4 weeks) and 15 gestational-age-matched normal pregnant women (35.5 ± 0.7 weeks) were included in the study. Mean NO level was significantly lower ($p < 0.001$) and mean Hcy level was significantly higher ($p < 0.001$) in the preeclamptic group. Elevated Hcy and oxygen free radical levels could decrease NO levels due to the reaction with each other and reduced NO may increase blood pressure and ischemia in preeclamptic patients. We have concluded that increased Hcy and oxygen free radical levels, and decreased NO levels are closely associated with preeclampsia-related endothelial dysfunction.

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Introduction

Preeclampsia is one of the most frequent complications of pregnancy, however, little is known about its etiology. Damage of the endothelial layer lining the blood vessel wall and reduced uteroplacental perfusion as a result of abnormal cytotrophoblast invasion of spiral arteries are considered to play an important role in the pathophysiology of preeclampsia. Placental ischemia/hypoxia is thought to lead to widespread activation/dysfunction of the maternal vascular endothelium which results in enhanced formation of endothelin, thromboxane and superoxide, increased vascular sensitivity to angiotensin II, and decreased formation of vasodilators such as nitric oxide and prostacyclin [1, 2].

Homocysteine (Hcy) results from the transmethylation of methionine and its metabolism depends primarily on three enzymes (methionine synthase, 5,10-methylenetetrahydrofolate reductase, cystathione β synthase) and several vitamin cofactors (vitamin B₆, vitamin B₁₂, folic acid). Genetic abnormality in these enzymes or deficiency of these vitamins leads to hyperhomocysteinemia (HHcy). HHcy is one of the congenital hypercoagulable states and a long-known vascular disease risk factor [3]. Nitric oxide (NO), a water and lipid-soluble free radical, is generated from *L*-arginine by the action of nitric oxide

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Table 1. Demographic data of the study participants

	Study cohort (n = 19)	Control cohort (n = 15)	p
Age, years	26.8 ± 1.3	25.9 ± 0.8	0.56
Gestational age, weeks	33.9 ± 1.4	35.5 ± 0.7	0.31
Parity	1.9 ± 0.4	1.4 ± 0.1	0.23
SBP, mm Hg	160.0 ± 4.1	116.8 ± 2.6	<0.0001
DBP, mm Hg	98.7 ± 2.3	73.5 ± 1.5	<0.0001
Proteinuria	2.3 ± 0.9	0.05 ± 0.01	0.0001

Numbers refer to mean ± SEM. Mean value for proteinuria changes from 1 to 4 positive due to dipstick measurements. SBP = Systolic blood pressure; DBP = diastolic blood pressure.

Table 2. Plasma homocysteine and NO measurements of the study participants (mean ± SD)

	Study cohort (n = 19)	Control cohort (n = 15)	p
Homocysteine, µmol/l	13.23 ± 6.76	4.86 ± 0.77	<0.001
Nitric oxide, µmol/l	46.41 ± 13.61	80.60 ± 12.69	<0.001

synthases and plays an important role in modulating blood flow and tissue injury in normal and several pathologic conditions [4]. Endothelial damage causes reduced NO release in preeclamptic patients.

The aim of this study was to show the difference in NO and Hcy levels in preeclamptic and normal pregnant women and then to relate HHcy and NO in pathophysiology of endothelial dysfunction seen in preeclampsia.

Material and Methods

Nineteen preeclamptic (group 1) and 15 gestational-age matched normal pregnant women (group 2) were recruited in the study. The study was approved by the local ethical committee of the university hospital and all patients provided informed consents. Gestational age was matched as ± 1–2 weeks according to the study group. Preeclampsia was diagnosed if the patients had blood pressure over 140/90 mm Hg on two or more occasions at least 4 h apart after the 20th week of gestation with proteinuria on a dipstick value of more than 1+ (30 mg/dl) on two separate occasions at least 6 h apart. Both mild and severe preeclamptic patients were included in the study. Blood was withdrawn from the study group immediately after the diagnosis before giving any medications and from normal pregnant women at their routine prenatal visits. None of the women in the study group was in labor. Blood plasma was separated and kept at –70 °C until

the day of measurement. All measurements were carried out in duplicate, and mean of the two measurements gave the final result. Plasma Hcy levels were detected by chemiluminescent method using an automatic immunoanalyser [Immuline-One].

Since plasma nitrite (NO₂) and nitrate (NO₃) levels can be used to estimate NO production, we measured the concentrations of these stable NO oxidative metabolites. Determination of NO₂ and NO₃ was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO₂ with a mixture of naphthylethylenediamine and sulfanilamide [5]. After samples were deproteinized with Somogyi reagent [6], an aliquot of the sample was mixed with fresh reagent. After 40 min incubation time the absorbance was measured in a spectrophotometer (Shimadzu UV-1201, Japan) to give the NO₂ concentration. A second aliquot was treated with copper-coated cadmium granules (Cd) in glycine buffer at pH 9.7 (2.5–3 g Cd granules for a 4-ml reaction mixture) to reduce NO₃ to NO₂. The concentration of NO₂ in this aliquot thus gave the total NO₃ plus NO₂, finally representing total NO concentration. A standard curve was established with a set of serial dilutions (100–5 µmol/l) of sodium nitrite. The resulting equation was then used to calculate the unknown sample concentrations.

Data were expressed as mean ± SD. Unpaired Student's t test was used to compare the homocysteine, NO and demographic data levels; proteinuria levels were compared with Mann-Whitney U test, p < 0.05 was accepted as significant.

Results

Demographic data of all patients are seen in table 1. There was no difference in the ages and gestational ages of the patients. The systolic and diastolic blood pressures and proteinuria levels were significantly higher in the preeclamptic group (p < 0.0001). Mean plasma Hcy and NO levels in patients with preeclampsia and controls are summarized in table 2. The Hcy level was significantly higher (p < 0.001), and NO level was significantly lower (p < 0.001) in the preeclamptic group.

Discussion

Previous studies have shown that oxidative stress in placental tissue is increased in preeclampsia and these free radicals play an important role in pathophysiology of the disorder, and evidence accumulates that oxidative stress is a mediator of endothelial dysfunction [7, 8]. In addition, other factors such as smoking, aging, environmental factors, toxic substance exposure, radiation increase oxidative stress which can be accepted as a criterion for tissue injury. NO levels increase during ischemic conditions. On the other hand, ischemia formed in the vicious cycle of preeclampsia produces more oxygen free radicals and aggravates endothelial dysfunction. Superox-

ide radical (O_2^-), which is generated by reperfusion and various factors, interacts with NO producing peroxy-nitrite ($ONOO^-$). Breakdown of NO by O_2^- anions may be a factor in reduced NO levels. Decreased release of nitric oxide by endothelial cells may in turn facilitate proliferation of vascular smooth muscle cells [14]. In our study, we found significantly lower NO levels in the preeclamptic patients. These findings may suggest that NO was degraded by O_2^- anions and peroxy-nitrite was formed in preeclampsia. As a result, increased oxygen free radicals may affect development of preeclampsia in two ways; first, a direct effect on cell and cell membranes which causes cell damage and atherosclerosis; second, vasoconstriction in vessels and increased ischemia in the placental bed by breakdown of NO.

Hcy is associated with several physiologic and life-style factors, including age, gender, blood pressure, serum cholesterol, smoking, alcohol and coffee consumption, physical activity, diet, and vitamin status [9]. Mild HHcy has been identified as a risk factor for arterial disease and venous thrombosis. Disturbances in the Hcy metabolism have also been reported as a risk factor for early pregnancy loss and for other congenital birth defects. However, besides embryonic or fetal consequences, HHcy has also been described as a cause of maternal obstetric complications such as preeclampsia [10]. In contrast, some authors cannot confirm mild HHcy as a risk factor [11]. In healthy human subjects, elevated Hcy concentrations are associated with impaired endothelium-dependent dilatation, an early manifestation of atherosclerosis. Conversely, lowering plasma Hcy concentrations with vitamin B treatment is associated with improved vascular endothelial function. Elevated levels of reduced but not oxidized Hcy promote endothelial injury and reacts with NO in the presence of oxygen to form S-nitrosomocysteine, which may decrease the bioactivity of NO due to newly formed stable nitrosothiol [12]. This reduced form promotes the generation of oxygen-derived free radicals via increases oxidized LDL [13]. In our study, Hcy levels in preeclamptic patients were significantly higher than the controls and this result would support the previous theory.

Sulfhydryl group of Hcy is believed to act catalytically with ferric or cupric ions in a mixed oxidation system to generate hydrogen peroxide, oxygen radicals and Hcy radicals [14]. Hydrogen peroxide, which freely passes cell membranes, is reduced and detoxified by glutathione peroxidase which is an important antioxidant enzyme in biologic systems. Homocysteine suppresses the expression of cellular glutathione peroxidase by endothelial cells [15]. High concentrations of Hcy increase intracellular reduced

Hcy which participates in the trans-sulfuration pathway and can replace cysteine in the synthesis of glutathione. Homocysteine decreases intracellular glutathione and NAD⁺ and the ratio between intracellular concentration of reduced and oxidized glutathione. Accumulation of hydrogen peroxides due to inefficiency of glutathione peroxidase is toxic in living organisms and may promote lipid peroxidation by oxygen radicals generated by Hcy.

An alternative hypothesis for HHcy-associated vascular disease is that HHcy may reflect inefficiency of intracellular methylation reactions, which is necessary for the normal repair of damaged proteins. Inefficient intracellular methylation may lead to persistence of damaged proteins, which in turn might be involved in atherosclerosis [15].

In conclusion, we have considered that HHcy, oxygen free radicals and NO can react with each other and are closely associated with endothelial dysfunction. According to our findings there is an important imbalance between NO production from dysfunctional endothelium and the Hcy level in preeclamptic women. HHcy and oxygen free radicals could increase arterial blood pressure by degradation and/or reduced expression and bioavailability of NO. Treatment with folic acid and antioxidants such as vitamin E and C may inhibit the development of HHcy and prevent increase in blood pressure.

References

- 1 Raijmakers MT, Zusterzeel PL, Steegers EA, Peters WH: Hyperhomocysteinaemia: A risk factor for preeclampsia? *Eur J Obstet Gynecol Reprod Biol* 2001;95:226–228.
- 2 Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA: Pathophysiology of preeclampsia: Linking placental ischemia/hypoxia with microvascular dysfunction. *Microcirculation* 2002;9:147–160.
- 3 Aubard Y, Darodes N, Cantaloube M: Hyperhomocysteinemia and pregnancy – Review of our present understanding and therapeutic implications. *Eur J Obstet Gynecol Reprod Biol* 2000;93:157–165.
- 4 Koltuksuz U, Irmak MK, Karaman A, Uz E, Var A, Özyurt H, Akyol Ö: Testicular nitric oxide levels after unilateral torsion/detorsion in rats pretreated with caffeic acid phenethyl ester. *Urol Res* 2000;28:360–363.
- 5 Cortas NK, Wakid WW: Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem* 1990;36:1440–1443.
- 6 Somogyi M: A method for the preparation of blood filtrates for the determination of sugar. *J Biol Chem* 1930;86:655.
- 7 Var A, Kuseu NK, Koyuncu F, Uyanik BS, Onur E, Yildirim Y, Oruc S: Atherogenic profile in preeclampsia. *Arch Gynecol Obstet* 2003;268:45–47.
- 8 Sikkema JM, van Rijn BB, Franx A, Bruinse HW, de Roos R, Stroes ES, van Faassen EE: Placental superoxide is increased in preeclampsia. *Placenta* 2001;22:304–308.
- 9 Ueland PM, Nygard O, Vollset SE, Refsum H: The hordoland homocysteine studies. *Lipids* 2001;36(suppl):S33–S39.
- 10 Nelen WL: Hyperhomocysteinaemia and human reproduction. *Clin Chem Lab Med* 2001;39:758–763.
- 11 Raijmakers MT, Zusterzeel PL, Steegers EA, Peters WH: Hyperhomocysteinaemia: A risk factor for preeclampsia? *Eur J Obstet Gynecol Reprod Biol* 2001;95:226–228.
- 12 Chambers CJ, Ueland PM, Wright M, Dore CJ: Investigation of relationship between reduced, oxidized, and protein-bound homocysteine and vascular endothelial function in healthy human subjects. *Circ Res* 2001;89:187–192.
- 13 Richards K, Katterhorn M, Donald A, Oakley G, Varghese Z, Rees L, Deanfield JE: Does oral folic acid lower total homocysteine levels and improve endothelial function in children with chronic renal failure? *Circulation* 2002;105:1810–1815.
- 14 Prasad K: Homocysteine, a risk factor for cardiovascular disease. *Int J Angiol* 1999;8:76–86.
- 15 Stehouwer CDA, Jacobs C: Abnormalities of vascular function in hyperhomocysteinaemia: Relationship to atherothrombotic disease. *Eur J Pediatr* 1998;157(suppl 2):107–111.