Plasma homocysteine and nitric oxide levels in preeclampsia

Preeklamside plasma homosistein ve nitric oksit seviyeleri

Ebru Erdemoğlu[1], Mustafa Uğur[1], Evrim Erdemoğlu[2]

[1]*Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health Research and Education Hospital, Ankara, Turkey*

[2]*Department Of Obstetrics And Gynecology, Turkish Military Ankara Mevki Soldier Hospital, Ankara, Turkey*

Abstract

Objective: Endothelial dysfunction, which is associated with decreased nitric oxide (NO) and hyperhomocysteinemia, is an essential component of preeclampsia Aim of our study was to investigate nitric oxide levels and homocysteine levels in preeclamptic women and to study the relationship between homocysteine and NO.

Material and Method: Thirty preeclamptic and thirty healthy pregnant women above 30 gestational weeks were included to study. Venous blood samples were collected from all women before administration of any drug. Homocysteine and two stable oxidation products of NO, nitrate and nitrite were analyzed in healthy pregnant women, mild preeclamptic, severe preeclamptic and eclamptic women.

Results: Demographical and obstetrical characteristics of patients were similar, except blood pressure measurements. Mean of homocysteine values were significantly higher in preeclamptic women (9.0±3.4 μmol/l) than healthy controls (5.3±1.3 μmol/l). Homocysteine levels were found to be increased as the severity of preeclampsia increased; with highest level in eclamptic patients (14.0±1.8 μmol/l, $p<0.001$). Cut-off values with good sensitivity and specificity was reproduced by ROC analysis. There was no significant difference in serum nitrite and nitrate levels. No association between hyperhomocysteinemia and NO was found.

Conclusions: Preeclampsia and severity of this pregnancy associated catastrophic disease is well-correlated with level of serum homocysteine. However, NO levels are not altered. Pathophsiology of hyperhomocysteinemia in preeclampsia probably does not involve impinging upon NO. (J Turkish-German Gynecol Assoc 2009; 10: 26-9)

Key words: Preeclampsia, eclampsia, nitric oxide, hyperhomocysteinemia

Özet

Amaç: Nitrik oksit azalması ve hiperhomosisteinemi ile ilişkili olan endotelial disfonksiyon preeklampsinin temel unsudur. Çalışmamızın amacı preeklamptik gebelerde nitrik oksit ve homosistein seviyelerini çalışmak, homosistein ile nitrik oksit arasındak ilişkiyi incelemekti.

Gereç ve Yöntemler: Çalışmaya 30. gebelik haftasından büyük eylemde olmayan 30 preeklamptik ve 30 sağlıklı gebe dahil edilmiştir. Venöz kanlar her hangi bir ilaç verimeden önce toplanmış. Homosistein ile nitrik oksitin iki kalıcı yıkım ürünü olan nitrit ve nitrat sağlıklı gebeler, hafif preeklamptik gebeler, şiddetli preeklamptik gebeler ve eklampsili gebelerde çalışılmıştır.

Bulgular: Kan basıncı ölçümleri dışında hastaların obstetrik ve demografik özellikleri benzerdi. Preeklamptik kadınların serum homosistein ortalaması (9.0±3.4 μmol/l) sağlıklı kontrollerden (5.3±1.3 μmol/l) daha yüksekti ($p<0.001$). Homosistein seviyeleri preeklampsinin şiddeti artıkça yükselmekteydi; en yüksek değerler eklampsili hastalarda (14.0±1.8 μmol/l, $p<0.001$) bulunmuştu. Homosistein değerleri için ROC analizinde sensitivite ve spesifitesi anlamlı olan eşik değer noktaları bulunmuştu. Serum nitrit ve nitrat seviyeleri gruplar arasında farklı değildi ($p>0.05$). Homosistein ve nitrik oksit seviyeleri arasında ilişki yoktu.

Sonuç: Preeklampsi ve şiddeti serum homosistein seviyeleriyle önemli derecede ilişkilidir. Ancak nitrik oksit seviyeleri preeklampsi de değişmemektedir. Hiperhomosisteineminin preeklampsi oluşturma patofizyolojisinde nitrik oksit muhtemelen yer almamaktadır.

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Anahtar kelimeler: Preeklampsi, eklampsi, nitric oksit, hiperhomosisteinemi

Introduction

Preeclampsia is characterized by hypertension and proteinuria after midgestation. Although many etiological factors and their possible mechanisms have been proposed, preeclampsia is still a disease of unknown cause (1). Managing and targeting therapy for preeclampsia are far from the underlying mechanisms and therefore it may fail. Recent evidence in literature focuses on endothelial dysfunction, nitric oxide (NO), and hyperhomocysteinemia. Results of reports of nitric oxide and preeclampsia are conflicting and it is reported that NO may not change, increase or decrease in preeclampsia (2). Homocysteine is currently related with vascular diseases and preeclampsia (3). High homocysteine levels may compromise release of endothelial NO or damage endothelial cell by oxidative stress leading to formation of thromboses (3,4). The aim of our study was to investigate NO levels and homocysteine levels in preeclamptic women and to study the association of homocysteine and NO.

Materials and Methods

Thirty preeclamptic and thirty healthy pregnant women above 30 gestational weeks were included in the study. Study was

Address for Correspondence / Yazışma Adresi: *Asst. Prof. Evrim Erdemoğlu, Süleyman Demirel Üniversitesi Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı 32260 Çünür, Isparta, Türkiye Phone: +90 246 211 21 44 Mobile: +90 505 272 43 44 e.mail: evrimmd@yahoo.com*

approved by local ethical committee. Each healthy pregnant women included in the study was at the same gestational age with preeclamptic women. All of the women included in the study had a first trimester ultrasonographic dating in accordance with their last menstrual period. Diagnosis of preeclampsia was based on the working group's report on high blood pressure in pregnancy (5). None of the subjects were in labor and women with superimposed preeclampsia, chronic hypertension, multiple gestation, and systemic disease were excluded from the study. Preeclamptic women with one of the following criteria (diastolic blood pressure higher than 110 mmHg, headache, cerebral disturbances, visual disturbances, epigastric pain, oliguria, proteinuria of 3+ or 4+ on semiquantative test, impaired liver function tests, thrombocytopenia, intrauterine growth retardation, pulmonary edema, and convulsions) were classified as severe preeclampsia (5). There were only 4 patients with eclampsia in the study.

Venous blood samples were collected from all women before administration of any drug. Peripheral blood samples were collected into EDTA containing tubes by venopuncture and the serums were prepared immediately by centrifugation at 3000 rpm for 15 minutes. Aliquots were stored at -80° celcus until further analysis.

The interaction of NO in a system is measured by the determination of both nitrate and nitrite concentrations in the sample. The relative levels of nitrite and nitrate can vary substantially; therefore the most accurate determination of total NO production requires quantization of both nitrate and nitrite. For this reason, the two stable oxidation products of NO, nitrate and nitrite, were assessed by a kit (Nitric oxide assay kit, Assay Designs Inc, Ann Arbor, MI, USA) involving the enzymatic conversion of nitrate to nitrite, by the enzyme nitrate reductase. Sulfanilamide containing Griess reagent I and N- (1-Naphthyl) etilenediamine containing Griess reagent II were used in nitrite assay procedure and nitrate assay procedure (total nitrite and nitrate). Standard curves for nitrite and then total nitrite + nitrate were obtained by measuring the optical density of each standard concentration at 540 nm. Nitrite assay procedure and measurements of optical density for nitrite was followed by nitrate procedure. After adding nitrate reductase enzyme dilution and 25 μl of NADH into all standard and sample wells for nitrate assay procedure, optical densities of each well was measured. These optical densities after nitrate assay indicated total nitrite and nitrate levels. Concentrations of nitrite and total nitrite+nitrate were extrapolated from graphs of Standard curves. Nitrate concentrations were calculated by subtracting nitrite from total nitrate and nitrite concentrations.

2-Mercaptoethylamine and the reducing agent, tris phosphine hydrochloride was added to samples for measurement of homocysteine. Protein is precipitated from solution and the thiol groups in the supernatant are then derivatized with a fluorescent thiol-specific dye. The fluorescent derivative mixture is then separated and the homocysteine concentration is automatically calculated by the analyzer (Drew Scientific Inc., U.K).

Statistics

Means of continuous variable data were analyzed by student-t test and OneWay ANOVA test. Categorical variables were analyzed by chi-square test. Pearson, Spearman correlation test, and regression tests were performed in order to analyze the correlation between homocysteine and NO levels. Cut-off values with sensitivity and specificity are calculated in ROC. The level of statistical significance was chosen to be $P < 0.05$. Statistical Analysis was performed using the SPSS 13.0 software program (SPSS, Chicago, IL, USA).

Results

Demographical and obstetrical characteristics of the patients were similar (Table 1). Forty-three percent of preeclamptic patients were classified as severe preeclampsia. Birthweight of babies of preeclamptic and healthy women were 2515±835 g and 3241±590 g, respectively ($p<0.001$). Male-female fetus ratio was similar in both groups. Mean of homocysteine levels in preeclamptic group was 9.0±3.4 μmol/l and mean of homocysteine values in normal pregnancies was 5.3±1.3 μmol/l ($p<0.0001$). Homocysteine values were significantly higher in severe preeclamptic patients than mild preeclamptic women. One-way ANOVA analysis of severe preeclamptic, mild preeclamptic, and normal pregnancies is shown in table 2. Homocysteine levels were found to be increased as the severity of preeclampsia increased (Figure 1). There were 4 patients (13.3%, 4/30) with eclampsia. Homocysteine levels of these eclamptic patients (14.0±1.8 μmol/l) were significantly higher from the other severe preeclamptic (11.8±3.2 μmol/l), mild preeclamptic (6.9 ± 1.4 μmol/l), and healthy pregnant (5.3 ±1.3 μmol/l, $p<0.001$).

Table 1. Demographical and obstetrical parameters of patients

Demographical and obstetric characteristics	Pregnancies		Statistical Significance
	Preeclamptic	Healthy	p value
Age	28±5.3	26.4±5.7	0.26
Gravid	2.7±1.7	2.1±1.0	0.12
Parity	1.2±1.5	0.6±0.7	0.06
Primigravid ratio	30% (n=9)	33% (n=10)	0.78
Systolic blood pressure (mmHg)	163.6±15.8	107.0±14.1	<0.001
Diastolic blood pressure (mmHg)	101.3±8.9	65.3±9.7	<0.001
Mean arteriel pressure (mmHg)	122.1±10.9	79.2±10.3	<0.001
Gestational Age	34.7±2.1	34.5±2.5	1.0

Table 2. Means of homocysteine levels in the healthy control group, mild preeclampsia and severe preeclampsia analyzed by one way-ANOVA. There are significant differences between mean of homocysteine in mild preeclamptic, severe preeclamptic and healthy control group

Homocysteine Values (A)	Homocysteine values (B)	Statistical Significance A-B (p)
Mild Preeclampsia 6.9 ± 1.4 μmol/l	Severe preeclampsia (11.8±3.2 μmol/l)	<0.001
	Healthy (5.3±1.3 μmol/l)	0.02

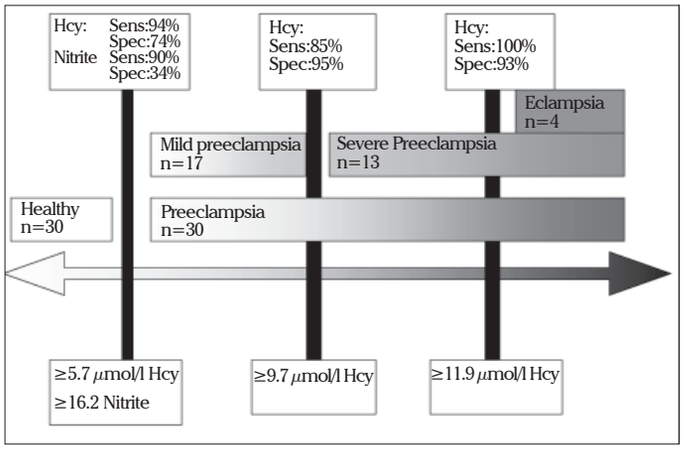

Figure 1. Homocysteine (Hcy) levels can divide patients into white zone (healthy), gray zone (mild preeclampsia), deep gray zone (severe preeclampsia) and extreme black zone (eclampsia). Although nitrite levels are of limited value, levels of total nitric oxide and nitrate are not statistically important

Means of total NO and nitrate in preeclamptic group were 73.3±27.8 μmol/l, 49.2±26.1 μmol/l, respectively. Means of total NO and nitrate in the control group were 63.8±16.9 μmol/l, 44.8±18.0 μmol/l, respectively (p>0.05). Mean of nitrite was significantly higher in preeclamptic women (24.1±8 μmol/l) than healthy controls (19.0±5.5 μmol/l, p=0.007). Neither severity of preeclampsia nor eclampsia was correlated with total NO, nitrate, and nitrite values.

The only significant factor affecting systolic, diastolic, and mean arterial pressure was homocysteine levels; increased homocysteine level was associated with increased blood pressure (p<0.05). Effect of increased homocysteine levels on NO was analyzed in regression analyses. No correlation was found between homocysteine levels, NO, and its' end-products.

Discussion

In the present study, it is found that homocysteine levels are increased in preeclamptic women. But NO levels are not different than healthy women and there is no correlation between homocysteine and NO levels.

It has been proposed that endothelial dysfunction is an essential component of preeclampsia (1,6) and reduced production of vasodilatory agents such as NO causes preeclampsia. However, there are reports of increased NO in preeclampsia (7-9). These studies suggest elevated NO production is adduced to physiological changes such as vasodilatation to overcome adverse placental effects. The present study failed to demonstrate any difference in serum nitrate, nitrite, and total NO levels and it is in accordance with previous reports which demonstrated no change in NO production (10,11).

Benedetto et al. reported that NO production is increased with severity of preeclampsia (11). However, in the present study, there was no difference between means of NO in healthy, mild, and severe preeclamptic patients.

NO has a multifunctional role in inflammation and various proinflammatory effects of NO have been described, including an increase in vascular permeability, cytotoxity and tissue damage, changes in glycosaminoglycan synthesis and apoptosis (12). There is evidence that NO participates in the regulation of uteroplacental and fetal blood flow, contributing to the control of basal vascular tone and attenuating the actions of vasoconstrictors (13). In vitro and in vivo studies provides information that NO plays a major role in the regulation of vascular tone control and placental perfusion in pregnancy (14,15).

Hyperhomocysteinemia is associated with vascular diseases such as hypertension, cerebrovascular accidents, peripheral vascular diseases, as well as early pregnancy loss and placenta abruption (3,16-18). Homocysteine levels are expected to be physiologically decreased in pregnancy due to increased glomerular filtration, increased plasma volume, and fetal usage. Homocysteine levels are reported to be higher in preeclampsia (15,18). Cotter et. al. reported that increased level of homocysteine in the second trimester of pregnancy can be used as a predictor of preeclampsia (18). The reason of high percentage of severe preeclamptic and eclamptic women in the present study was because of the status of our hospital as a reference center in the city and the region. Plasma homocysteine concentration was found to be correlated with the severity of preeclampsia in the present study. However, more studies are needed to document the effect of high homocysteine levels on preeclampsia and eclampsia. There were only 4 eclamptic patients in the study and this was one of the main limiting factors. However, the statistical analysis of this eclamptic group cannot be validated by this study and further studies with large number of eclamptic patients and intrauterine growth retardation is needed.

It is proposed that hyperhomocysteinemia can damage endothelial cells by oxidative stress or can directly impinge on NO metabolism (3,4). Homocysteine and NO are mutually interacting. It is reported that high levels of homocysteine can impair the function of eNOS and decrease NO levels (20). Shukla et al. reported that homocysteine decreased NO production in rats by forming superoxides (21). On the other hand, NO inhibits methionine synthetase, which has a critical role in homocysteine metabolism (22). It could be hypothesized that increased homocysteine levels can cause decreased NO production. In the present study, there was no correlation between homocysteine levels and NO levels.

As a conclusion, hyperhomocysteinemia could be associated with preeclampsia and severity of preeclampsia. Levels of homocysteine could be used to assess the severity of preeclampsia in addition to other clinical and laboratory measures. Pathophsiology of hyperhomocysteinemia in preeclampsia probably does

not involve NO production. NO levels were not altered in preeclamptic patients in the present study. More studies are needed to elucidate the pathophysiology of hyperhomocysteinemia in preeclampsia.

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