Maternal Serum Concentrations of Metabolites of Nitric Oxide in Preeclampsia

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Abstract

Objective: The purpose of this study was to determine the serum levels of nitric oxide (NO) metabolites in pregnant women with preeclampsia and to find out any relation between the pathogenesis of preeclampsia and NO levels.

Methods: In this prospective, case-control study, venous blood samples were collected from pregnant women with preeclampsia (n=30) and age-matched healthy pregnant women (n=30). The serum fractions of these samples were assayed for total nitrite/nitrate levels. The outcomes of the pregnancies were evaluated and groups were compared to each other for the clinical characteristics and NO metabolites. Student's paired t-test and $\chi 2$ test were used for the statistical analysis. P value less than 0.05 was considered statistically significant.

Results: The mean total serum levels of nitrite/nitrate in pregnant women with preeclampsia and healthy pregnant women were 59.0 ± 15.55 µmol/L and 41.0 ± 10.37 µmol/L respectively. There were significantly higher total nitrite/nitrate levels in the maternal serum of preeclamptic women ($p<0.05$). Also, significantly higher nitrite/nitrate levels were found in severe preeclamptic women compared with those of mild preeclamptic and healthy pregnant women ($p<0.05$).

Conclusion: Maternal serum levels of NO metabolites were higher in pregnant women with preeclampsia and it was directly related with the severity of the disease. This may be a compensatory mechanism to increase the blood flow to uteroplacental unit in preeclampsia.

Keywords: Pregnancy, nitric oxide, preeclampsia, nitrite, nitrate.

Preeklampsili gebelerde maternal serum nitrik oksit metabolitlerinin seviyeleri

Amaç: Bu çalışmanın amacı, preeklamptik gebelerdeki nitrik oksit (NO) metabolitlerinin serum seviyelerini tespit etmek ve NO seviyesi ile preeklampsi arasında bir ilişki olup olmadığını ortaya çıkartmaktır.

Yöntem: Bu prospektif, vaka kontrollü çalışmada preeklampsi tanısı konulmuş gebe kadınlardan (n=30) ve benzer yaş grubundaki sağlıklı gebelerden venöz kan örnekleri alındı. Alınan kan örneklerinin serum fraksiyonlarında toplam nitrit/nitrat konsantrasyonu hesaplandı. Daha sonra her iki grupdaki gebeliklerin sonuçları araştırıldı ve iki grup klinik özellikleri ile NO metabolitleri açısından birbirleri ile karşılaştırıldı. İstatistiksel değerlendirme için eşli t-test ve χ^2 test kullanıldı. 0.05'den küçük p değeri istatistiksel olarak anlamlı kabul edildi.

Bulgular: Preeklamptik ve sağlıklı gebelerde ortalama toplam nitrit/nitrat seviyeleri sırasıyla 59.0 ± 15.55 µmol/L ve 41.0 ± 10.37 µmol/L olarak bulundu. Preeklamptik gebelerin maternal serumlarında toplam nitrit/nitrat seviyeleri anlamlı derecede daha yüksekti ($p<0.05$). Ayrıca şiddetli preeklampsi tanısı alan gebelerdeki nitrit/nitrat seviyeleri, hafif preeklamptik ve sağlıklı gebelerle karşılaştırıldığında, anlamlı derecede yüksek saptandı ($p<0.05$).

Sonuç: Bu çalışmada NO metabolitlerinin maternal serum seviyeleri preeklamptik gebelerde daha yüksek saptandı ve bu yükseklik preeklampsinin şiddeti ile doğru orantılı olarak bulundu. Bu artış, preeklampside uteroplasental kan akımını artırmaya yönelik kompensatuar bir mekanizma olabilir.

Anahtar Sözcükler: Gebelik, nitrik oksit, preeklampsi, nitrit, nitrat.

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Introduction

Preeclampsia is one of the most common health problems experienced by pregnant women and it appears in approximately 5-8% of pregnancies later than 20 weeks.[1] This disease which is characterized by hypertension, edema and proteinuria is a very important pregnancy complication which leads to both fetal and maternal morbidity and mortality by affecting all systems in the body. Even though etiology and pathogenesis of preeclampsia could not been clarified definitely until now, it is considered that the basic problem is decreased placental bleeding appeared due to abnormal cytotrophoblast invasion and extensive endothelium damage appeared dependent on this.[2] While sensitivity of blood walls to angiotensin II with production of substances like this "endothelin" and "thromboxane" as a result of endothelium damage, it was indicated that production of vasodilator substances like nitric oxide (NO) and prostacyclin decreased.[3]

NO which is synthesized from L-arginine, which is an amino acid, by the effect of "nitrite oxide synthase (NOS) enzyme is an effective free radical which inhibits thrombocyte aggression and leads to vasodilatation in vessels.[4] This very little lipophilic molecule which was defined as "endothelium derived relaxing factor (EDRF)"[5] in first years it was founded but then it was understood that this is NO[6,7] increases cyclic guanozine monophostate (cGMP) concentration by activating sithozolic guanilate cyclasis and thus leads to vasodilatation by decreasing Ca++ level in-cells. NO is a gaseous molecule, half-life of it is so short (nearly 4 seconds) and rapidly converts into its metabolites nitrite (NO_2) and nitrate (NO_3).[8] For this reason, in majority of studies in which NO production is researched concentration of its metabolites nitrite and nitrate was measured in different samples (maternal plasma, serum, urine and various tissues, amniotic fluid, placenta, umbilical venous blood, cerebrospinal fluid).

It is known that both NO production and response to NO increase in normal pregnancy[4,8] and it is though that this increase plays a role in many physiological mechanisms which provide continuation of the pregnancy.[9] For this reason, there may be a dysfunction appear in NO system in pathogenesis of preeclampsia and many studies have been held in this issue for recent 10 years.[10] However, incompatible results were presented in these studies held, and it is reported that NO level increases in some pregnant women with preeclampsia,[11,15] decreases in some of them,[16,18] and does not change in others.[19,21]

This study was planned with the aim of exhibiting whether or not changes in nitric oxide level play a role in pathogenesis of preeclampsia, and it does what is the relationship between severity of preeclampsia and nitric oxide levels, and total serum nitrite nitrate levels were researched in cases included in the study for this purpose.

Methods

30 pregnant women among those who applied high-risk pregnancy polyclinic of our hospital and diagnosed as preeclampsia and 30 pregnant women as control group among those who applied antenatal polyclinic and any problem was not be determined and who have similar characteristics with study group were included in this study. Control group was formed among primigravid pregnant women who have applied our polyclinic by matching with pregnant women with preeclampsia in terms of age, pregnancy week and risk factors. The study was planned in accordance with principles of Helsinki declaration and permit document for study was obtained from ethic committee of our hospital and "informed approval" document was taken from people who participated into the study. All pregnant women included in the study composed of primigravid women who have completed 28th week of their pregnancy but have not had a pregnancy later than 36th week, between 20 and 35 ages, do not have therapy pregnancy, smoking habit or another diagnosed systemic disease, risk factors like (diabetes mellitus, chronic hypertension, auto-immune diseases, chronic kidney failures, urinary infection, cardiovascular diseases, thyroid dysfunction, infective diseases etc.), do not take any medical treatment other than iron and folic acid, for whom any fetal anomaly was not diagnosed and have monomer pregnancy.

ACOG criteria were used for preeclampsia diagnosis.[1] Accordingly tension arterial value, measured in sitting position at least for two times after a 15 minute rest and in 6 hour intervals even though normotensive is known before 20th week of pregnancy, being higher than systolic 140 mmHg and diastolic 90 mmHg and determining proteinuria higher than 300mg in 24 hour urine was considered as preeclampsia. In case tension arterial value

measured in the same conditions is systolic 160 mmHg, diastolic 110 mmHg, more than 5 g proteinuria is determined in 24-hour urine, urine outflow lesser than 500 ml in 24 hour, fetal growing retardation, thrill or kidney dysfunction is determined "severe preeclampsia" was diagnosed.

For all pregnant women included in study and control groups full blood, full urine, kidney function tests, liver function tests, preprandial blood sugar, viral hepatitis indicators, indirect Coombs test, protein in 24-hour urine, creatine, urine culture examinations and fundus examination were held. In addition fetal monitor, ultrasonography and fetal Doppler researches were held in all of pregnant women in order to determine the situation of fetal well-being. A diet lack of nitrite and nitrate (which does not include spinach, beet and cooked meat) was given to all pregnant women included in the study for 24 hour and peripheral venous blood was drawn following an 8-hour hunger for the purpose of determining serum total nitrite/nitrate value before starting any treatment for preeclampsia. Serum fractions of drawn blood were separated and preserved at 70°C in order to research on mass after the end of study period.

Measurement of serum total nitrite/nitrate levels was performed by a technician who is not informed about the study after all pregnant women had procreated. The method described by Cortas and Wakid was used in measurement of serum total nitrite/nitrate levels.[22] This method is based on principle of degradation of nitrate to nitrite through copper covered cadmium granules and measurement of obtained nitrite together with existing nitrite in the atmosphere. The method of standard adding was used in order to remove effect of inhibitors like ascorbat etc.

All data in the study were given as ± standard deviation. Statistical evaluations were conducted by using SPSS Ver. 11.0 (Chicago, IL, ABD) program, and independent groups with t-test ki square test, $p<0.05$ was considered statistically meaningful.

Results

30 pregnant women with preeclampsia 10 among which was diagnosed as slight, 20 as severe preeclampsia and 30 healthy pregnant women as control groups were included in the study. When general characteristics of pregnant women included in the study were decided, a significant difference cannot be determined between pregnant group with preeclampsia and healthy pregnant women included in control group in terms of pregnancy week in which samples were taken for control of age and nitrite/nitrate serum levels ($p>0.05$). Nonetheless, average systolic and diastolic tension arterial values and protein amount determined in 24-hour urine were founded significant high in pregnant women with preeclampsia who form study group ($p<0.05$). Pregnancy period in pregnant women with preeclampsia and average birth weight of newborn babies were also founded significant lower compare to control group ($p<0.05$). In addition, 63.3% caesarean rates in study group was determined significant higher compare to control group in which this rate is 33.3% ($p<0.05$). While perinatal in one pregnant and postnatal fetal loss in one pregnant was observed in the study group, no fetal loss was observed in pregnant women included in control group. General characteristics of pregnant women included in the study and differences between the groups were presented in Table 1.

Maternal serum average nitrite/nitrate level in pregnant women with preeclampsia who form study group was founded as 59.0 ± 15.55 µmol/L. this values was determined as 41.0 ± 10.37 µmol/L in the control group and there was a statistically significant difference between two groups ($p<0.05$) (Table 2). When mild and severe preeclamptic pregnant women were evaluated separately maternal serum average nitrite/nitrate values were determined respectively as 51.8±13.63 µmol/L and 73.8±6.0 µmol/L. The value determined in the group with severe preeclampsia was a significant higher value statistically compare to both the group with mild preeclampsia and the control group ($p<0.05$).

Discussion

In many studies conducted until now it was declared that NO production increases at least in vessel bed and uterus during pregnancy period and this increase ensures uterus to keep silent by decreasing contractility until accouchement activity starts and plays an important role in adaptation of vascular structures in pregnancy.[4,8] In researches conducted for this purpose, it was indicated that cGMP and nitrite/nitrate amount in plasma and urine in normal pregnancies and thus NO produc-

Table 1. General characteristics of pregnant women included in the study.

Characteristic	Preeclampsia (n=30)	Normal pregnancy (n=30)	P value
Age (year) (Average±SD)	29.06±5.40	28.06±4.01	ad
Pregnancy week when the sample is taken(Average±SD)	32.6±2.8	32.1±3.0	ad
Pregnancy week in accouchement (Average±SD)	35.2±2.7	38.1±2.1	<0.05
Systolic blood pressure (mmHg) (Average±SD)	153±19	114±8	<0.05
Diastolic blood pressure (mmHg) (Average±SD)	113±14	73±6	<0.05
Protein in 24-hour urine (g) (Average±SD)	4.28±1.02	0.18±0.22	<0.05
Birth weight (g) (Average±SD)	2488±751	3226±276	<0.05
Cesarian accouchement rate	19 (%63.3)	10 (%33.3)	<0.05
Perinetal death	2 (%6.66)	0	ns

SD: Standard deviation
ns: Not significant

Table 2. Maternal serum nitrite/nitrate concentrations.

	Maternal serum average nitrite/nitrate amount (µmol/L)
Preeclampsia (n=30)	59.0±15.55a
Mild preeclampsia (n=10)	51.8±13.63a
Severe preeclampsia (n=20)	73.8±6.0a,b
Normal pregnancy (n=30)	41.0±10.37
P value	<0.05

Note: Values are presented as average±standard deviation.
a: Significant compare to control group (p<0.05)
b: Significant compare to mild preeclamptic group (p<0.05)

tion also increases.[9] Setting out from this point the idea that there may be a decrease in NO production in explanation of clinic findings appeared in pregnant women with preeclampsia unlike normal pregnant women was come up and for this purpose tens of studies were conducted and published oriented to exhibit possible role of NO system in preeclampsia.[10-21] In majority of these studies NO_2 and/or NO_3 levels were searched rather than NO half-life of which is so short in maternal and fetal plasma.[11-14,16-21] In addition to this, some studies have been published in which NO metabolites in amnious fluid,[23] brain-spinal cord fluid[24] or cord blood[12,15] of pregnant women with preeclampsia were searched. As a result of these studies incompatible results were reported and even though it was exhibited that NO system may play a role in pathogenesis of preeclampsia, it was not clarified how this occurs. For this purpose in studies recently held with higher-techniques NOS isoenzymes which take a role in NO synthesis were researched rather than NO metabolites, but again different results have been reported and it was declared that NOS mRNA production decreases[25] or does not change in pregnant women with preeclampsia[26] or there is no difference in NOS isoenzyme levels which is examined with immune dying between preeclamptic and normotensive pregnant women.[27]

In our study on the other hand, maternal serum average nitrite/nitrate levels in preeclamptic pregnant women were found meaningfully high compare to normotensive healthy pregnant women. When the effects of NO is taken into consideration it is expected that decrease in NO efficiency in a preeclamptic pregnant and vasoconstriction appear dependent on this should play a role in pathogenesis of preeclampsia, on the contrary increase in NO metabolites makes us to think that NO system does not have a direct role on pathogenesis of preeclampsia, rather this is a compensator mechanism oriented to decrease emerged preeclampsia findings and increase utero-placental blood stream. This result also complies with some studies held before.[11-14] In addition higher NO metabolite levels found in severe preeclampsia group in our

study can be considered as a finding which supports this thought. Similarly, Pathak et al have founded serum nitrite/nitrate levels in preeclamptic pregnant women significant higher compare to normotensive pregnant women in their study and declared that they determined direct proportion between the severity of preeclampsia and nitrite/nitrate levels.[28] Shaamash et al have also indicated in their study held in 2000 there is a positive correlation between the severity of preeclampsia and the amount in maternal fetal circulation of NO metabolites.[12] Even in a study, which declared that any difference was not detected between preeclamptic pregnant women and normotensive ones in terms of serum nitrite/nitrate levels, NO production was found higher in severe preeclampsia group.[21] All these findings are findings which support the result determined in our population and which indicates that the higher the severity of preeclampsia the higher NO production occurs.

It has been maintained that this increase in NO production and oscillation occur depending on endothelium damage and oxidative stress appear in preeclamptic pregnant women, and it was also thought that thrombocytes, trophoblasts, desidual and myometrial cells may also be reasons of increased NO production.[13] Excess NO secreted from these sources may be a compensator mechanism oriented to increase vasoconstriction, thrombocyte aggression appear in pregnant women and uteroplacental blood stream decreased as a result of this. It was contended that pregnant women with preeclampsia pass through four different periods theoretically and according to this hypothesis it was affirmed that the period in which any finding has not been detected before estimated factor emerged constitutes the first phase, the period in which any clinic finding has not emerged since the balance was continued with compensator mechanisms after the estimated factor emerged constitutes the second phase, the period in which severe findings have not been determined since compensator mechanisms were still effective even though mild clinic findings emerged constitutes the third phase, and the period in which compensator mechanisms were not sufficient any more and severe clinic findings have been determined constitutes the fourth phase.[8] According to hypothesis bases of compensator mechanisms in the second and third phases are formed by NO system, after possible factor which leads to preeclampsia emerges NO production and oscillation starts to increase and it prevents severe findings to emerge until last phases of pregnancy, however this cannot be sufficient after a certain stage even though NO levels are so high and then picture of severe preeclampsia emerges. The findings obtained in our study also support this hypothesis.

Conclusion

In this study total nitrite/nitrate levels in maternal serums of preeclamptic pregnant women were found meaningfully high compare to normotensive healthy pregnant women. This high level increase in direct proportion with severity of preeclampsia. In preeclampsia which is one of the most serious problems appear in pregnant women NO system may play a preventive role directed to increase utero-placental blood stream. However, studies which can directly indicate NO levels in different periods of pregnancy are required in order to determine the definite role of NO in preeclampsia pathogenesis.

References

1. American College of Obstetricians and Gynecologists. Diagnosis and management of preeclampsia and eclampsia. ACOG Practice Bulletin No. 33. Washington D.C.: ACOG; 2002.
2. Granger JP, Alexander BT, Llinas MT, Bennett WA, Khalil RA. Pathophysiology of hypertension during preeclampsia linking placental ischemia with endothelial dysfunction. *Hypertension* 2001; 38: 718-22.
3. Granger JP, Alexander BT, Bennett WA, Khalil RA. Pathophysiology of pregnancy-induced hypertension. *Am J Hypertens* 2001;14: 178S-85S.
4. Weiner CP, Thompson LP. Nitric oxide and pregnancy. *Semin Perinatol* 1997; 21(5): 367-80.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288(5789): 373-6.
6. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 1987; 84(24): 9265-9.
7. Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327(6122): 524-6.
8. Buhimschi IA, Saade GR, Chwalisz K, Garfield RE. The nitric oxide pathway in pre-eclampsia: pathophysiological implications. *Hum Reprod Update* 1998; 4(1): 25-42.
9. Rosselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. *Hum Reprod Update* 1998; 4(1): 3-24.

10. Lowe DT. Nitric oxide dysfunction in the pathophysiology of preeclampsia. *Nitric Oxide* 2000; 4(4): 441-58.
11. Ranta V, Viinikka L, Halmesmaki E, Ylikorkala O. Nitric oxide production with preeclampsia. *Obstet Gynecol* 1999; 93(3): 442-5.
12. Shaamash AH, Elsnosy ED, Makhlouf AM, Zakhari MM, Ibrahim OA, EL-dien HM. Maternal and fetal serum nitric oxide (NO) concentrations in normal pregnancy, pre-eclampsia and eclampsia. *Int J Gynaecol Obstet* 2000; 68(3): 207-14.
13. Yoneyama Y, Suzuki S, Sawa R, Miura A, Doi D, Otsubo Y, Araki T. Plasma nitric oxide levels and the expression of P-selectin on platelets in preeclampsia. *Am J Obstet Gynecol* 2002;187(3): 676-80.
14. D'Anna R, Baviera G, Corrado F, Crisafulli A, Ientile R, Buemi M, Squadrito F. Neurokinin B and nitric oxide plasma levels in pre-eclampsia and isolated intrauterine growth restriction. *BJOG* 2004; 111(10): 1046-50.
15. Karabulut AB, Öztürk LÇ, Sezgin N, Hasçalık Ş, Kafkaslı A. Preeklamptik gebe kadınlarda ve bebeklerinin kordon kanında, nitrik oksit metabolitleri olan nitrit ve nitratın plazma düzeylerinin araştırılması. *İnönü Üniversitesi Tıp Fakültesi Dergisi* 2001; 8(1):1-4.
16. Mutlu-Turkoglu U, Aykac-Toker G, Ibrahimoglu L, Ademoglu E, Uysal M. Plasma nitric oxide metabolites and lipid peroxide levels in preeclamptic pregnant women before and after delivery. *Gynecol Obstet Invest* 1999; 48(4): 247-50.
17. Aydin S, Benian A, Madazli R, Uludag S, Uzun H, Kaya S. Plasma malondialdehyde, superoxide dismutase, sE-selectin, fibronectin, endothelin-1 and nitric oxide levels in women with preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2004; 113(1): 21-5.
18. Müderris İİ, Özçelik B, Muhtaroğlu S. Preeklampsi ve eklampside maternal serum nitrik oksid ve total antioksidan aktivite seviyelerinin önemi. *Türkiye Klinikleri Jinekoloji Obstetrik Dergisi* 2002; 12(1): 25-9.
19. Egerman RS, Andersen RN, Manejwala FM, Sibai BM. Neuropeptide Y and nitrite levels in preeclamptic and normotensive gravid women. *Am J Obstet Gynecol* 1999; 181: 921-3.
20. Pasaoglu H, Bulduk G, Ogus E, Pasaoglu A, Onalan G. Nitric oxide, lipid peroxides, and uric acid levels in pre-eclampsia and eclampsia. *Toboku J Exp Med* 2004; 202(2): 87-92.
21. Diejomaoh FM, Omu AE, Al-Busiri N, Taher S, Al-Othman S, Fatinikun T, Fernandes S. Nitric oxide production is not altered in preeclampsia. *Arch Gynecol Obstet* 2004; 269(4): 237-43.
22. Cortas NK, Wakid NW. Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. *Clin Chem* 1990; 36(8 Pt 1): 1440-3.
23. Tranquilli AL, Bezzeccheri V, Giannubilo SR, Scagnoli C, Mazzanti L, Garzetti GG. Amniotic vascular endothelial growth factor (VEGF) and nitric oxide (NO) in women with subsequent preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2004; 113(1): 17-20.
24. Celik O, Hascalik S, Turkoz Y, Hascalik M, Gokdeniz R. Cerebrospinal fluid nitric oxide level changes in preeclampsia. *Eur J Obstet Gynecol Reprod Biol* 2003; 111 (2): 141-5.
25. Wang Y, Gu Y, Zhang Y, Lewis DF. Evidence of endothelial dysfunction in preeclampsia: decreased endothelial nitric oxide synthase expression is associated with increased cell permeability in endothelial cells from preeclampsia. Am J Obstet Gynecol 2004; 190(3): 817-24.
26. Wang X, Athayde N, Trudinger B. Maternal plasma from pregnant women with umblical placental vascular disease does not affect endothelial cell mRNA expression of nitric oxide synthase. J Soc Gynecol Investig 2004; 11(3): 149-53.
27. Rajagopal M, Moodley J, Chetty R. Nitric oxide: does it have an etiological role in pre-eclampsia? Acta Obstet Gynecol Scand 2003; 82(3): 216-9.
28. Pathak N, Sawhney H, Vasishta K, Majumdar S. Estimation of oxidative products of nitric oxide (nitrates, nitrites) in preeclampsia. Aust NZJ Obstet Gynaecol 1999; 39(4): 484-7.