Serum neutrophil gelatinase associated lipocalin and plasma nitric oxide levels in healthy and preeclamptic pregnants

N. Dogan[1], S. Yildirmak[1], V. Mihmanli[2], M. Vardar[1], Y.G. Ozbanazi[1], M. Cakmak[3], F. Sezgin[4]

[1]Okmeydani Educational and Research Hospital, Department of Clinical Biochemistry, Istanbul
[2]Okmeydani Educational and Research Hospital, Department of Obstetric and Gynecology, Istanbul
[3]Gulkent State Hospital, Department of Clinical Biochemistry, Isparta
[4]Istanbul University, Department of Industrial Engeneering, Istanbul (Turkey)

Summary

Aims: The authors aimed to evaluate serum neutrophil gelatinase associated lipocalin (NGAL) and plasma nitric oxide (NO) levels in preeclamptic and healthy pregnant women above 24 gestation weeks. *Materials and Methods:* Forty-nine healthy and 21 preeclamptic (total 70) pregnant women participated voluntarily in the study. Presence of 140 mmHg and above systolic and 90 mmHg and above diastolic blood pressure which emerges after 20th gestation week, proteinuria more than 300 mg/24 hour, and edema were used as diagnostic criterion for preeclamptic pregnant women. Measurements of serum NGAL and plasma NO were performed with enzyme linked immunosorbent assay (ELISA) and photometric method, respectively. *Results:* Serum NGAL and plasma NO levels of healthy and preeclamptic groups did not show a statistical difference. In preeclamptic group, a statistically meaningful correlation was found between level of NGAL and body mass index (BMI) of sampling time, creatinine and NGAL, total protein and NO, and albumin and NO. *Conclusions:* Serum NGAL levels, correlated with serum creatinine levels in this study, may be the early marker of renal damage which may develop mainly due to inflammation and endothelial damage. The authors could not find a statistical difference for serum NGAL and plasma NO levels between healthy pregnant and preeclamptic groups. Varieties peculiar to humans in preeclampsia, impossibility of obtaining first trimester tissue material as an evidence of inadequate trophoblast invasion, and different appearance of maternal reaction to underlying main pathology in every case may restrict clarification of etiopathogenesis.

Key words: Neutrophil gelatinase associated lipocalin; Nitric oxide; Preeclampsia.

Introduction

Although preeclampsia is one of the most serious complications of pregnancy affecting 8% of whole pregnancies, only little is known regarding its etiology [1]. Endothelial damage occurs both in the early and late onset preeclampsia and it is responsible of hypertension and proteinuria [2]. It is accepted that disordered early trophoblastic invasion, decreased placental perfusion, placental ischemia, oxidative stress cascade, and consequently disordered placental factors (imbalance in angiogenic and prothrombotic factors) playing a key role in inducing maternal endothelial dysfunction are effective in pathophysiology [3]. It is assumed that in the center of pathophysiology there are inflammation and endothelial damage [4,5].

Neutrophil gelatinase associated lipocalin (NGAL) is a glycoprotein with a weight of 25 kDa which was first detected in granules of human neutrophils [6]. It plays roles of growth and differentiation factors in many cell types [7]. In studies it was observed that it was induced strongly during inflammation [8]. It was reported that NGAL, inhibiting inactivation of matrix metalloproteinase-9 (MMP-9), causes accelerated proteolytic activity which accompanies prolonged activity on collagen degradation and increased angiogenesis [6,9].

Nitric oxide (NO) which is produced by nitric oxide synthase (NOS) enzyme from L –arginine is a strong endogen vasodilator molecule playing an important role in regulating blood stream and blood pressure in pregnancy. NO inhibits thrombocyte aggregation, leukocyte adhesion to vascular endothelia, and proliferation of smooth muscle cells. It has been claimed that the decrease in bioavailability of NO might be responsible for biologic collocation of preecclampsia and endothelial dysfunction and at least homocystein, asymmetric dimethyl arginine (ADMA), and NO could be considered partially responsible of preeclampsia etiology and they could be taken into account as indicator of severity of disease [10].

In the present study the authors aimed to detect serum NGAL and NO levels and correlation between them in preecclamptic gravids having a gestational age older than 24 weeks (second and third trimester) and in healthy individuals ended up with a healthy delivery having same gestational age.

Materials and Methods

This study was performed in Okmeydani Educational and Research hospital between April 2010 and September 2010. Forty-nine healthy gravids between ages of 18-40 years and 21 gravids with preeclampsia between 18-40 years participated in the study. The ethic approval was taken in ethic council of H.M. Sisli Etfal Educational and Research hospital with decision of number 86 in 01.12.2010.

Revised manuscript accepted for publication November 20, 2013

Table 1. — *Comparison of demographic and clinical findings in healthy and preeclamptic gravid groups.*

	Healthy group (n=49) mean±SD median (min-max)	Preeclamptic group (n=21) mean±SD median (min-max)	*p*
Age	26.0 (18.0-40.0)	31.90±7.11	0.003
Height (cm)	161.33±5.76	160.0 (150.0-175.0)	0.762
Pre-pregnancy weight (kg)	59.0 (43.0-93.0)	65.24±13.46	0.223
Pre-pregnancy BMI	23 (17.60-36.40)	24.73-4.73	0.249
Weight (at sampling) (kg)	67.92±10.32	77.62±13.20	0.002
BMI (at sampling)	26.11±4.0	29.50±4.90	0.003
Gestational age (at sampling)	26.0 (24.0-28.0)	32.43±4.13	<0.0001
Systolic blood pressure (mmHg)	110.0 (100.0-130.0)	150.0 (140.0-180.0)	<0.0001
Diastolic blood pressure (mmHg)	70.0 (50.0-80.0)	100.0 (70.0-120.0)	<0.0001
Number of pregnancies	2.0 (1.0-5.0)	2.0 (1.0-5.0)	0.215
Gestational age at delivery	39.0 (37.0-40.0)	34.0 (28.0-39.0)	<0.0001
Infant birth weight (g)	3300 (2500-3800)	1808.09±929.94	<0.0001

Table 2. — *Comparison of laboratory findings in healthy and precclamptic gravid groups.*

	Healthy group (n=49) mean±SD median (min-max)	Preeclamptic group (n=21) mean±SD median (min-max)	*p*
Glucose (mg/dl)	74.0 (61.0-108.0)	97.0 (60.0-285.0)	<0.0001
Urea (mg/dl)	14.0 (8.0-25.0)	24.0 (12.0 -42.0)	<0.0001
Creatinin (mg/dl)	0.60 (0.30-0.90)	0.80 (0.50-1.40)	<0.0001
T. Chol (mg/dl)	248.71 ± 36.89	291.57 ± 82.24	0.031
TG (mg/dl)	195.0 (89.0-478.0)	283.0 (124.0-602.0)	0.001
HDL-C (mg/dl)	65.0 (38.0-126.0)	76.29±16.41	0.030
LDL-C (mg/dl)	136.06±31.08	146.0 (90.0-320.0)	0.082
AST (U/l)	19.43±5.38	27.0 (14.0-346.0)	0.012
ALT (U/l)	13.0 (3.0-49.0)	20.0 (5.0-235.0)	0.029
ALP (U/l)	73.0 (37.0-156.0)	155.71±79.45	<0.0001
GGT (U/l)	9.0 (4.0-34.0)	12.0 (4.0-139.0)	0.032
LDH (U/l)	197.76±31.15	235.0 (131.0-983.0)	0.018
T. Protein (g/dl)	6.7 (5.60-7.80)	6.02±0.56	<0.0001
Albumin (g/dl)	3.5 (3.10-4.0)	3.0 (2.10-3.40)	<0.0001
T. Bil. (mg/dl)	0.31 (0.11-0.76)	0.41 (0.17-2.12)	0.110
D. Bil. (mg/dl)	0.06 (0.02-0.25)	0.05 (0.01-0.53)	0.536
Na (mmol/l)	137.51±2.43	138.0 (132.0-141.0)	0.751
K (mmol/l)	4.0 (3.50-4.70)	4.2 (3.50-5.0)	0.085
TSH (mIU/ml)	2.07±1.03	2.12 (0.08-13.09)	0.604
NGAL (ng/ml)	124.68 (72.42-218.82)	120.44±50.88	0.078
NO (μM)	39.83±15.84	37.07±14.48	0.496

Systolic blood pressure of 140 mmHg or above, diastolic blood pressure of 90 mmHg or above, existence of protein over 300 mg in 24 hour urine, and edema after 20th week of pregnancies were defined as preeclampsia. With the patients previously having known blood pressures, increases over 30 mmHg in systolic and 15 mmHg in diastolic blood pressures were evaluated as significant for preeclampsia diagnosis. Precclamptic gravids were selected according to this criteria. Healthy controls were selected among second term gravids having normal pregnancy control values and in 50 g oral glucose tolerance test (OGTT) serum glucose levels 70-105 mg/dl in zero minutes and below 140 mg/dl in 60 minutes.

Multiple pregnancies, vegetarianism, smoking habit, alcohol consumption, diabetes mellitus, thyroid function disorder, hepatic and cardiac diseases were considered as excluding criteria from the study.

Blood samples of healthy and preeclamptic gravids were obtained after 12-hour fasting for routine biochemical tests and protein in spot urine were evaluated with urine strips. Ten ml of blood was obtained to vacuum tube with gel for routine biochemical parameters and NGAL. Tube with gel was centrifugated after clot was formed in 4,000 rpm for ten minutes and serum was separated. Biochemical parameters and thyroid stimulating hormones (TSH) were analyzed on the same day. Undiluted serum samples were stored in – 80°C for NGAL measurement. Five ml blood was taken to tube with EDTA for NO measurement, centrifuged in 4,000 rpm for ten minutes and plasma was separated. Undiluted plasma samples were stored in – 80°C for NO measurement. Repeating freezing and thawing were not performed and samples were analyzed within six months.

Serum glucose, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gama glutamyl transferase (GGT), total bilirubin, direct bilirubin, sodium, potassium, total protein, albumin, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) triglyceride tests of control and patient group were analyzed photometrically in an autoanalyzer; TSH in an autoanalyzer; protein in spot urine full automatic urine autoanalyzer with urine strips; NGAL with enzyme linked immunosorbent assay (ELISA) method and NO was measured with enzymatic colorimetric method. LDL–C levels were calculated with Friedewald Formula.

SPSS 17.0 package program was used for statistical analysis of data in this study. Primarily it was searched whether the variants were scattered normally in bases of groups as healthy and preeclamptic. In accordance of this search, in healthy group. Height when sample was obtained weight; when sample was obtained body mass index (BMI), total cholesterol, LDL-C, Na, TSH, NO; in case of preeclamptic group, age, weight before pregnancy, BMI before pregnancy, weight when sample was obtained, BMI when sample was obtained, gestational age when sample was obtained, baby weight during delivery, urea, total cholesterol, triglyceride, HDL-C, ALP, total protein, urine protein, NGAL, and NO variants showed normal distribution. Remaining variants were not in compliance with normal distribution, this was detected by Kolmogorov-Smirnov normality test. Necessary test statistics were obtained by taking account of this property. The results were in level of 95% reliance and significance was evaluated as $p < 0,05$.

Results

Demographic and clinical findings of healthy and preeclamptic groups are shown in Table 1 and laboratory findings in Table 2.

Table 3. — *Correlations between NGAL and NO levels with demographic and clinical findings in healthy and preeclamptic gravid groups.*

	Healthy group				Preeclamptic group			
	NGAL	*p*	NO	*p*	NGAL	*p*	NO	*p*
Systolic B.P.	0.061	0.675	-0.028	0.850	0.303	0.181	-0.319	0.158
Diastolic B.P.	0.071	0.628	-0.106	0.470	0.048	0.836	0.036	0.877
Pre-pregnancy BMI	0.103	0.481	0.436	0.002	-0.431	0.051	-0.090	0.697
BMI at sampling	0.064	0.663	0.380	0.007	-0.445	0.043	-0.165	0.474
Infant birth weight	0.231	0.110	0.196	0.178	-0.278	0.223	0.097	0.676
Maternal age	0.075	0.607	0.148	0.312	-0.007	0.974	0.090	0.697
Number of pregnancies	0.014	0.925	0.225	0.120	-0.251	0.273	-0.249	0.276
Gestational age at delivery	0.113	0.441	-0.042	0.773	-0.349	0.121	-0.064	0.783
Gestational age at sampling	-0.171	0.240	-0.127	0.385	-0.186	0.419	-0.138	0.550
Protein in spot urine (mg)	-	-	-	-	0.240	0.295	-0.154	0.506

B.P.: Blood pressure

Table 4. — *Correlations that was founded meaningful between NGAL and NO levels with other laboratory findings in healthy and precclamptic gravid groups.*

	Healthy group				Preeclamptic group			
	NGAL	*p*	NO	*p*	NGAL	*p*	NO	*p*
Creatinin (mg/dl)	0.09	0.537	0.055	0.706	0.521	0.015	0.260	0.254
T. protein (g/dl)	-0.198	0.173	0.165	0.258	0.307	0.176	0.546	0.010
Albumin (g/dl)	-0.146	0.318	-0.064	0.66	0.220	0.338	0.544	0.011
Na (mmol/l)	-0.418	0.003	-0.037	0.803	0.029	0.900	-0.002	0.993

Correlations between NGAL and NO levels with demographic and clinical findings in healthy and preeclamptic gravid groups are shown in Table 3.

Correlation coefficients that were founded meaningful between NGAL and NO levels with other laboratory findings in healthy and precclamptic gravid groups are shown in Table 4. A statistically significant correlation between Na and NGAL variants were detected in healthy group. A statistically significant correlation between creatinine and NGAL; T. protein and NO; albumin and NO were detected in preeclamptic group.

Discussion

Although preeclampsia is one of the most serious complications of pregnancy affecting 8% of whole pregnancies, only a few is known about its etiology [1].

In a study in which NGAL and pregnancy associated plasma protein A (PAPP-A), which are assumed as determinants of endothelial and placental damage, were evaluated in trimester preeclampsia and serum median NGAL levels and were detected higher in preeclamptic group compared to control group and first trimester NGAL sensitivity was found lower than second trimester NGAL sensitivity in estimating late onset preeclampsia. Additionally in this study NGAL levels were found positively correlated with systolic and diastolic blood pressures and proteinuria [2].

Elneihoum *et al.* [11] in a study, detecting serum NGAL levels were correlated with increased blood pressure; this result was reported as consistent with a previous study in which NGAL levels were detected higher in non-pregnant hypertensive women in comparison to healthy controls and correlated positively with diastolic blood pressure.

In the study of D'Anna *et al.* [12], serum NGAL levels were detected higher in second trimester of preeclamptic gravids than that of normal gravids and a positive and high correlation between blood pressure and proteinuria with NGAL was observed in preeclamptic group.

Arikan *et al.* [13], detected rather lower plasma lipocalin-2 levels in preeclamptic gravids than healthy gravid group and they reported that this findings can display the role of lipocalin-2 in pathogenesis of preeclampsia.

D'Anna *et al.* [2], serum median NGAL levels were detected higher in preeclampsia than control group and in predicting late onset preeclampsia, although the sensitivity of first trimester NGAL was detected lower than that of second trimester NGAL, it was reported that first trimester serum NGAL was an early indicator of late onset preeclampsia.

In the present study there was no difference between healthy and preeclamptic gravids in terms of serum NGAL levels.

D'Anna *et al.* [12] detected a significant and positive correlation between NGAL and proteinuria in preeclamptic gravids. However, while the present authors could not find a correlation between serum NGAL and protein levels in spot urine in preeclamptic group, they detected a significant

positive correlation between serum creatinine and serum NGAL levels. On the other hand, serum creatinine levels were detected significantly higher in preeclamptic group than healthy group. This finding is consistent with that of Nickolas *et al.* [7] who claimed serum NGAL levels were early indicators of renal damage, therefore serum NGAL levels correlated with serum creatinine in preeclamptic gravids could be early indicators of renal damage.

Seligman *et al.* [14] performed a study in 26 preeclamptic and 26 normotensive gravids to search the role of NO in preeclampsia. They detected significantly lower serum nitrite and nitrate levels in preeclamptic gravids than control group. Similarly, Li *et al.* [15] studied cGMP levels that is the second messenger for NO. When compared to control group, nitrite, nitrate and cGMP levels in preeclamptic gravids were significantly lower.

Mao D *et al.* [10] reported they detected significantly lower plasma NO levels in preeclamptic gravids in comparison to healthy gravids. However in some studies concerning preeclamptic gravids, conflicting results which were higher, lower and unchanged were obtained [14, 16, 17].

As a result the present authors did not observe significant differences between preeclamptic and healthy gravids terms of NGAL and NO levels. The studies concerning this issue have been conflicting results. The studies performed with larger preeclamptic gravid sampling groups are needed.

References

[1] Sibai B., Dekker G., Kupferminc M.: "Pre-eclampsia". *Lancet*, 2005, *365*, 785.

[2] D'Anna R., Baviera G., Giordano D., Russo S., Dugo N., Santamaria A., Corrado F.: "First trimester serum PAPP-A and NGAL in the prediction of late-onset pre-eclampsia". *Prenat. Diagn.*, 2009, *29*, 1066.

[3] Redman C.W., Sargent I.L.: "Latest advances in understanding preeclampsia". *Science*, 2005, *308*, 1592.

[4] Holthe M.R., Staff A.C., Berge L.N., Fagerhol M.K., Lyberg T.: "Calprotectin plasma level is elevated in preeclampsia". *Acta Obstet. Gynecol. Scand.*, 2005, *84*, 151.

[5] Brodszki J., Länne T., Laurini R., Strevens H., Wide-Swensson D., Marsál K.: "Vascular mechanical properties and endothelial function in pre-eclampsia with special reference to bilateral uterine artery notch". *Acta Obstet. Gynecol. Scand.*, 2008, *87*, 154.

[6] Kjeldsen L., Bainton D.F., Sengeløv H., Borregaard N.: "Identification of neutrophil gelatinase-associated lipocalin as a novel matrix protein of specific granules in human neutrophils". *Blood*, 1994, *83*, 799.

[7] Nickolas T.L., O'Rourke M.J., Yang J., Sise M.E. Canetta P.A., Barasch N., *et al.*: "Sensitivity and specificity of a single emergency department measurement of urinary neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury". *Ann. Intern. Med.*, 2008, *148*, 810.

[8] Cowland J.B., Sørensen O.E., Sehested M., Borregaard N.: "Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alfa". *J. Immunol.*, 2003, *171*, 6630.

[9] Fernandez C.A., Yan L., Louis G., Yang J., Kutok J.L., Moses M.A.: "The matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex plays a role in breast tumor growth and is present in the urine of breast cancer patients". *Clin. Cancer Res.*, 2005, *11*, 5390.

[10] Mao D., Che J., Li K., Han S., Yue Q., Zhu L., *et al.*: "Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia". *Arch. Gynecol. Obstet.*, 2010, *282*, 371.

[11] Elneihoum A.M., Falke P., Hedblad B., Lindgärde F., Ohlsson K.: "Leukocyte activation in atherosclerosis: correlation with risk factors". *Atherosclerosis*, 1997, *131*, 79.

[12] D'Anna R., Baviera G., Giordano D., Todarello G., Corrado F., Buemi M.: "Second trimester neutrophil gelatinase-associated lipocalin as a potential prediagnostic marker of preeclampsia". *Acta Obstet. Gynecol.*, 2008, *87*, 1370.

[13] Arikan C., Özkaya M., Adali E., Kilinc M., Coskun A., Ozer A., Bilge F.: "Plasma lipocalin-2 levels in pregnant women with preeclampsia, and their relation with severity of disease". *J. Matern. Fetal Neonatal Med.*, 2010, *24*, 291.

[14] Seligman S.P., Buyon J.P., Clancy R.M., Young B.K., Abramson S.B.: "The role of nitric oxide in the pathogenesis of preeclampsia". *Am. J. Obstet. Gynecol.*, 1994, *171*, 944.

[15] Li L., Chen Z., Xu Y.: "The changes of plasma nitric oxide patients with pregnancy induced hypertension". *Chin. J. Obstet. Gynecol.*, 1996, *31*, 454.

[16] Smárason A.K., Allman K.G., Young D., Redman C.W.: "Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with preeclampsia". *Br. J. Obstet. Gynecol.*, 1997, *104*, 538.

[17] Silver R.K., Kupfermic M.J., Russell T.L.: "Evaluation of nitric oxide as a mediator of severe preeclampsia". *Am. J. Obstet. Gynecol.*, 1996, *175*, 1013.

Address reprint requests to:
S. YILDIRMAK, M.D.
Department of Clinical Biochemistry
Okmeydani Educational and Research Hospital
Darulaceze Cad. No. 25
Istanbul (Turkey)
e-mail: yildirmaksembol@gmail.com