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Circulating Levels of Neutrophil Gelatinase–Associated Lipocalin (NGAL) Correlate With the Presence and Severity of Preeclampsia

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Abstract

Objective: Neutrophil gelatinase–associated lipocalin (NGAL) has emerged as a reliable marker of acute renal injury and is produced at the maternal–fetal interface but its role in preeclampsia has not been systematically examined. This study investigated whether plasma NGAL concentrations changed in patients with preeclampsia at diagnosis compared to normotensive controls. **Study Design:** A case–control study was performed. Plasma was collected from women with preeclampsia and normotensive controls matched for age, gestational age, and body mass index. Plasma NGAL concentrations were measured by specific enzyme-linked immunosorbent assay. **Results:** Patients with preeclampsia had significantly higher NGAL concentrations than controls (median [range]: 203.8 ng/mL [66.1-575.4] vs 122.8 ng/mL [7.0-669.7]; $P = .047$). In subgroup analysis, patients with severe preeclampsia had significantly higher NGAL concentrations than those with mild preeclampsia. Plasma NGAL concentrations were positively correlated with the amount of proteinuria in women with preeclampsia ($P = .003$). **Conclusions:** Plasma NGAL concentrations were significantly elevated in women with preeclampsia versus normotensive controls, and concentrations appear to be associated with the severity of the disease.

Keywords
NGAL, preeclampsia, plasma, biomarker, pregnancy

Introduction

Preeclampsia (gestational proteinuric hypertension) complicates 6% to 8% of all pregnancies, and is a major cause of maternal/perinatal morbidity and mortality.[1] It cannot be accurately predicted or prevented, and the only effective treatment is delivery. As such, early and accurate diagnosis is critical to optimize pregnancy outcome. Recent studies have shown that a number of biomarkers may be associated with preeclampsia, including several proangiogenic (vascular endothelial growth factor, placental growth factor) and antiangiogenic factors (soluble fms-like tyrosine kinase 1, soluble endoglin).[2–13] We propose that neutrophil gelatinase-associated lipocalin (NGAL) may be another such biomarker.

The NGAL is a 25-kDa lipocalin that was originally purified from activated human neutrophils, but has since been shown to be produced by other immune cells, hepatocytes, adipocytes, and renal tubular cells. The NGAL appears to serve primarily as a transport shuttle transferring small molecules (including retinoids, arachidonic acid, prostaglandins, fatty acids, steroids, iron, and matrix metalloproteinases [MMPs]) in a safe and controlled fashion between adjacent cells.[14-17] It has also been shown to be a critical component of innate immunity to bacterial infection, a function that appears to be related to its ability to transport and sequester iron into immune cells,[18] and has been associated also with cellular growth and differentiation.[19,20] The NGAL concentrations are low in healthy human tissues (such as kidney, lung, stomach, liver, and colon), but appear to be upregulated in pathologic conditions following epithelial cell injury.[21,22] Several reports suggest a relationship between NGAL and pelvic inflammatory disease,[23] chronic obstructive pulmonary disease,[24] and atherosclerosis.[25] The NGAL expression has also been found in malignancies of the breast, lung,

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Table 1. Clinical Characteristics of the Study Population.

	Control (n = 37)	Preeclampsia (n = 37)	P
Maternal age,[a] years	33.0 (18-43)	33.0 (15-41)	.999
Nulliparity, n	16 (43.2%)	27 (73.0%)	.018
Prepregnancy body mass index,[a] kg/m^2	21.1 (15.2-26.4)	22.0 (17.7-31.7)	.412
Gestational age at sampling,[a] weeks	37.0 (24.1-40.4)	34.0 (26.0-39.9)	.052
Gestational age at delivery,[a] weeks	37.7 (34.0-41.3)	34.9 (28.0-40.0)	.001
Birth weight,[a] g	2815 (2110-3820)	2150 (800-4320)	<.001

[a] Data are given as median (range).

colon, and pancreas,[26–28] where it appears to be associated with tumor progression since overexpression of NGAL enhances the enzymatic activity of MMP-9 which, in turn, leads to lysis of basal membranes and extracellular matrix.[29–31] The NGAL is not expressed in normal ovaries but is upregulated in ovarian cancer cells.[21,32]

More recently, NGAL has emerged as a potentially useful diagnostic biomarker for acute kidney injury,[33–38] and several studies have reported that elevated NGAL concentration in plasma and/or urine is superior to conventional biomarkers in the prediction of acute kidney injury.[38–40] Despite the central role of the kidney in the pathogenesis of preeclampsia and the observation that NGAL is produced at the maternal–fetal interface,[41] there have been few studies about the systemic role of NGAL in preeclampsia. In the previous studies, serum NGAL concentrations significantly increased in women who subsequently developed preeclampsia.[42,43] The studies showed that NGAL could be used in the prediction of preeclampsia before the clinical diagnosis of the disease. However, comparison of NGAL levels according to the disease severity was not performed in those studies.

The pathogenesis of preeclampsia is not completely understood, but likely involves a series of complex mechanisms including incomplete invasion and remodeling of the maternal spiral arteries,[44] high circulating levels of antiangiogenic factors and proinflammatory cytokines,[2,45] and generalized endothelial cell dysfunction.[46–49] It is promising therefore to posit that the generalized endothelial injury associated with preeclampsia could lead to an upregulation of circulating NGAL levels. This study examined whether plasma NGAL concentrations changed in patients with preeclampsia at diagnosis compared to normotensive controls.

Materials and Methods

Study Population

A case–control study was performed. The study population consisted of women with a singleton pregnancy admitted to Seoul National University Hospital between January 2009 and March 2011. Patients with preeclampsia were selected as cases. Preeclampsia was defined as new-onset hypertension (systolic blood pressure $\geq$140 mm Hg and/or diastolic pressure $\geq$90 mm Hg on 2 occasions at least 4 hours but no more than 7 days apart) and significant proteinuria ($\geq$300 mg/24 h or $\geq$1+ on dipstick) after 20 weeks of gestation in a previously normotensive woman.[1] A diagnosis of severe preeclampsia was based on the presence of preeclampsia and one or more of the following criteria: persistent systolic blood pressure $\geq$160 mm Hg or diastolic blood pressure $\geq$110 mm Hg, proteinuria $\geq$5 g in a 24-hour urine collection or $\geq$3+ dipsick in random urine samples, oliguria (<500 mL/d), cerebral or visual disturbances, pulmonary edema, epigastric or right upper-quadrant pain, impaired liver function, thrombocytopenia (<100 000 platelets/μL), or fetal growth restriction.[1] An equal number of normotensive women with a singleton pregnancy admitted in the same period were selected as controls. Age, gestational age at sampling and body mass index (BMI) were considered for the selection of controls. Women with other medical or obstetric complications (such as chronic hypertension, diabetes, systemic lupus erythematosus, acute systemic inflammation, fever, or preterm labor) or with major congenital fetal anomaly were excluded from both groups.

Sample Collection

Maternal blood samples were collected after obtaining written informed consent. The local institutional review board at Seoul National University Hospital approved the study. Maternal venous blood was obtained at the time of hospital admission and centrifuged, and the plasma was stored at −70°C.

NGAL Immunoassay

Plasma NGAL concentrations were measured using a commercially available enzyme-linked immunosorbent assay (Bioporto, Gentofte, Denmark).[50] Both intra- and interassay coefficients of variation were <10%. All measurements were performed twice, and the result reported as the average of the 2 values.

Statistical Analysis

Nonparametric techniques were used for statistical analysis. Comparison of the continuous variables was performed using the Mann-Whitney *U* test. Proportions were compared using Pearson chi-square or Fisher exact test. The nonparametric statistical dependence between 2 variables was measured by Spearman's rho.

Results

Study Population

Demographic and clinical characteristics of the case and control groups (n = 37 for each) are summarized in Table 1. Of the 37 women with preeclampsia, 25 had severe preeclampsia and 12 had mild preeclampsia. There were no statistical differences

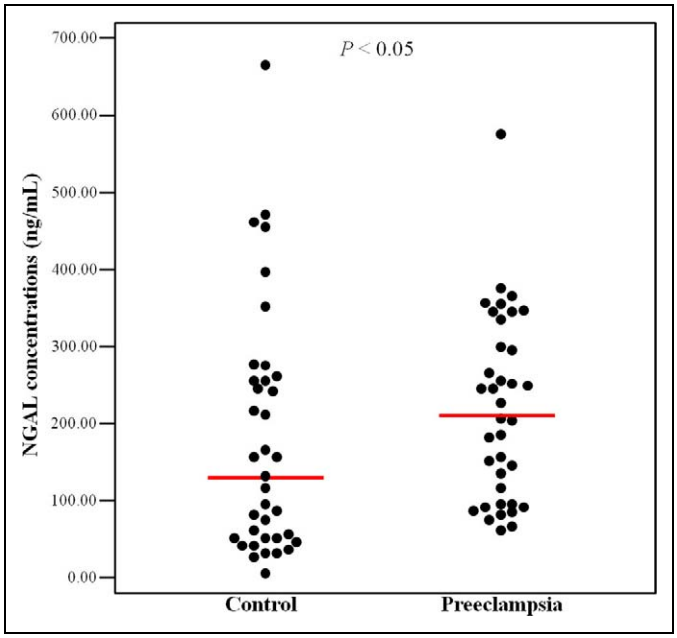

Figure 1. Neutrophil gelatinase–associated lipocalin (NGAL) concentrations in women with/without preeclampsia. The median plasma NGAL concentrations in women with preeclampsia was significantly higher than that of controls (median [range]: 203.77 ng/mL [66.1-575.4] vs 122.77 ng/mL [7.0-669.7], $P = .047$ by Mann-Whitney *U* test).

between the women with and without preeclampsia in terms of maternal age, prepregnancy BMI, and gestational age at sampling. However, compared to normotensive controls, women with preeclampsia were significantly more likely to be nulliparous, has significantly lower median gestational age at delivery, and a significantly lower median birth weight (Table 1).

Plasma NGAL Concentrations in Women With/Without Preeclampsia

Patients with preeclampsia had significantly higher circulating NGAL concentrations than normotensive controls (median [range]: 203.8 ng/mL [66.1-575.4] vs 122.8 ng/mL [7.0-669.7], $P = .047$, Figure 1).

Plasma NGAL Concentrations in Women With Preeclampsia According to the Severity

In subgroup analysis, patients with severe preeclampsia had a significantly lower gestational age at sampling (median [range]: 31.9 weeks [26.0-38.1] vs 38.4 weeks [33.7-39.9], $P < .001$) and lower gestational age at delivery (median [range]: 33.4 weeks [28.0-38.6] vs 38.8 weeks [34.9-40.0], $P < .001$) than those with mild preeclampsia, but there were no statistical differences according to the severity of preeclampsia in terms of maternal age, parity and prepregnancy BMI (Table 2). Patients with severe preeclampsia had significantly higher median NGAL concentrations than those with mild preeclampsia (median [range]: 237.5 ng/mL [67.4-575.4] vs 125.9 ng/mL [66.1-295.7], $P = .049$, Figure 2). The plasma level of NGAL was significantly higher in patients with hypertension of severe criteria (systolic blood pressure $\geq$160 mm Hg or diastolic blood pressure $\geq$110 mm Hg) than those without severe hypertension ($P = .024$). This association could not be accounted for by the difference in gestational age, since plasma NGAL concentrations were not affected by gestational age at sampling in the study population as a whole ($P = .630$) or in the subgroup analysis ($P = .476$). There was also no significant difference in the median plasma NGAL concentrations between nulliparous and multiparous women ($P = .097$). And there were no statistical correlations between the NGAL concentrations and maternal white blood cell count, platelet count, or gestational age at delivery.

Table 2. Clinical Characteristics of Women With Preeclampsia According to Severity.

	Severe Preeclampsia (n = 25)	Mild Preeclampsia (n = 12)	*P*
Maternal age,[a] years	34.0 (15-39)	33.0 (28-41)	.643
Nulliparity, n	17 (68.0%)	10 (83.3%)	.445
Prepregnancy body mass index,[a] kg/m^2	20.0 (17.7-25.5)	22.7 (19.0-31.7)	.110
Gestational age at sampling,[a] weeks	31.9 (26.0-38.1)	38.4 (33.7-39.9)	<.001
Gestational age at delivery,[a] weeks	33.4 (28.0-38.6)	38.8 (34.9-40.0)	<.001
Birth weight,[a] g	1560 (800-2690)	2815 (2060-4320)	<.001

[a] Data are given as median (range).

Correlation Between Plasma NGAL Concentrations and Proteinuria

Plasma NGAL concentrations were positively correlated with the amount of proteinuria in women with preeclampsia ($r = .485$, $P = .003$). One patient failed to complete a 24-hour urine collection because she required an emergency cesarean delivery for worsening preeclampsia shortly after admission; she was excluded from this analysis. Of note, her urinary albumin was 4+ on dipstick and the level of plasma NGAL concentration was high (367.6 ng/mL). There were 4 patients with severe preeclampsia who had relatively low levels of proteinuria (300-500 mg/24 h). Of these 4 patients, 3 women had plasma NGAL concentrations <100 ng/mL. The fourth woman had a high plasma NGAL concentration (327 ng/mL) and she went on to develop significant proteinuria (5796 mg/24 h) 1 month later. Serum creatinine concentrations showed a tendency toward a positive correlation with plasma NGAL concentrations in women with preeclampsia but this association failed to reach statistical significance ($r = .328$, $P = .055$).

Plasma NGAL Concentrations and Fetal Growth

Thirty-six percent (9 of 25) of patients with severe preeclampsia delivered neonates with a birth weight of <10th percentile

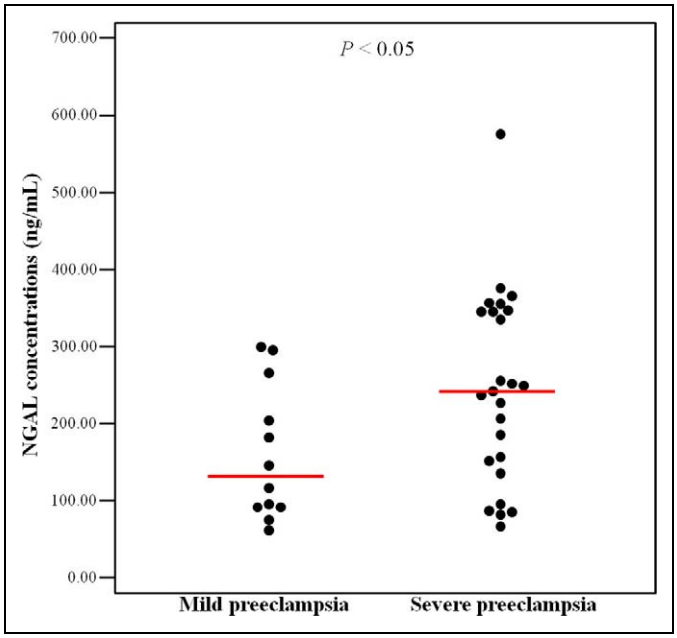

Figure 2. Neutrophil gelatinase–associated lipocalin (NGAL) concentrations in women with mild and severe preeclampsia. The median plasma NGAL concentrations in women with severe preeclampsia was significantly higher than that in women with mild preeclampsia (median [range]: 237.5 ng/mL [67.4-575.4] vs 125.9 ng/mL [66.1-295.7], $P = .049$ by Mann-Whitney *U* test).

for gestational age. These 9 women had a wide range of plasma NGAL concentrations (from 80.7 to 575.4 ng/mL). There was no significant difference in the median plasma NGAL concentrations between women with severe preeclampsia who delivered small-for-gestational age fetuses and those who did not (median [range]: 212.8 ng/mL [80.7-575.4] vs 240.4 ng/mL [67.4-378.3], $P = .718$). There were no statistical correlations between the NGAL concentrations and birth weight.

Discussion

The Principal Findings of the Study

The principal findings of the study include (1) that patients with preeclampsia had significantly higher circulating NGAL concentrations than gestational age-matched normotensive controls; (2) that patients with severe preeclampsia had significantly higher NGAL concentrations than those with mild preeclampsia; and (3) that plasma NGAL concentrations were positively correlated with the amount of proteinuria in women with preeclampsia. We conclude, therefore, that there is an association between plasma NGAL concentrations and preeclampsia and that circulating NGAL levels may reflect the severity of the disease. The inability to clearly separate mild from severe preeclampsia can be explained by the underlying heterogeneity of preeclampsia with its complex pathophysiology.

A positive correlation between plasma NGAL concentration and degree of proteinuria is consistent with prior observations suggesting that NGAL is a reliable marker of acute renal injury.[33–38] In addition, although it failed to reach statistical significance, there did appear to be a trend toward a positive correlation between serum creatinine and plasma NGAL concentrations in women with preeclampsia.

Previous studies showed the relationship between serum NGAL concentration and preeclampsia before the onset of the disease development for prediction of the preeclampsia.[42,43,51,52] On the other hand, we analyzed the association between plasma NGAL concentration and preeclampsia at the time of diagnosis, moreover, we identified NGAL levels based on the severity of preeclampsia.

NGAL in Pathogenesis of Preeclampsia

The elevated concentrations of NGAL in maternal blood with preeclampsia likely represent a consequence of the generalized endothelial dysfunction that characterizes this disease as has been previously proposed.[42,43] However, analysis of NGAL concentration according to the severity of preeclampsia or relationship between the concentrations of NGAL and serum creatinine had not been performed in the previous studies. Preeclampsia results from incomplete invasion of the spiral arteries by extravillous cytotrophoblasts. The resultant defective implantation results in a failure of the trophoblast to remodel the maternal vasculature and the persistence of the maternal spiral arteries as small-caliber high-resistance vessels.[53–57] Various antiangiogenic factors and proinflammatory mediators then act to aggravate the underlying endothelial cell injury in women who subsequently develop preeclampsia. This generalized endothelial cell injury leads, in turn, to increased levels of NGAL in the maternal circulation. The NGAL was originally identified as a glycoprotein in human neutrophils that complexes with MMP-9,[19,20,23] and its expression is upregulated in a number of inflammatory conditions with underlying endothelial cell injury.[23–25,35–39] In preeclampsia, defective implantation results in oxidative stress and the upregulation of antiangiogenic factors and proinflammatory mediators in the maternal circulation leading to a variety of clinical symptoms. Therefore, it is reasonable to assume that NGAL may be associated with pathogenesis of preeclampsia.

Weaknesses of the Study and Proposals for Future Research

This study was limited by a relatively small number of participants and the difficulty of identifying adequate normotensive controls, especially at early gestational ages. There were women who delivered at preterm in the control group, however, their causes of preterm delivery were not concerned with the conditions such as acute systemic inflammation, fever, or preterm labor. They delivered at preterm by cesarean section or vaginal delivery after induction of labor because of maternal underlying kyphosis, isolated decreased amniotic fluid volume without fetal growth restriction, vaginal bleeding, or preterm premature rupture of membrane without uterine contractions.

There was no growth restricted fetus in the control group. Even though it is not certain that the causes of preterm delivery are associated with elevated NGAL levels and most of the cases had low NGAL concentrations, the poor diagnostic performance of plasma NGAL for preeclampsia in our study might come from the limitation of controls. A prospective study with larger number of samples would be required to confirm and further validate the results of this study. On the other hand, several women of the normotensive group in this study had unexplained elevated plasma NGAL levels without any medical or obstetrical problem, which did not appear to adversely affect pregnancy outcome.

In summary, NGAL concentrations were significantly increased in the circulation of women with preeclampsia as compared with gestational age-matched normotensive controls, and the degree of NGAL elevation appear to be associated with the severity of preeclampsia. Additional studies are needed to further investigate the role of this biomarker in the diagnosis and prediction of preeclampsia.

Authors' Note

This study was presented at the 32nd Annual Meeting of the Society for Maternal–Fetal Medicine, Dallas, Texas (February 11, 2012).

Declaration of Conflicting Interests

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