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Original Research

Neutrophil gelatinase associated lipocalin-2 (Ngal) levels in preeclampsia

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Summary

Objectives: Lipocalin-2 (LCN-2) is an immune modulator. It is highly associated with inflammation, ischemia, neoplastic invasion, and transformation. Preeclampsia (PE) is a pregnancy-related disease resulting from the incomplete invasion of trophoblasts. Endothelial cytokines and inflammation have crucial roles in the pathogenesis of PE. We aimed to investigate serum LCN-2 levels in pregnant women with either no PE, mild PE or severe PE. Furthermore, we determined how LCN-2 levels relate to findings of Doppler ultrasound of the arteries in these patients. *Material and Methods*: Pregnant women with severe PE (n = 51), mild PE (n = 27), or no PE (n = 42) were involved in the study. Serum LCN-2 levels and Doppler ultrasonography (USG) evaluation were performed at the time of diagnosis of PE or in the cases of uncomplicated pregnancy, just prior to delivery. Women with non-complicated pregnancies were followed up for an additional 8 weeks after delivery. Intrauterine growth restriction (IUGR) was evaluated according to Alexander curve references. *Results*: Serum LCN-2 levels were significantly higher in pregnant women with PE. Higher LCN-2 levels were found in association with abnormal uterine blood flow and IUGR. Mean gestational age was lower in preeclamptic pregnancies and associated with high serum levels of LCN-2. *Conclusion*: PE is one of the most prevalent causes of pregnancy-associated complications. Early diagnosis and management of the disease are crucial. The level of serum LCN-2 may provide additional prognostic value along with other clinical and laboratory features of the disease.

Key words: Lipocalin-2; Preeclampsia; Pregnancy.

Introduction

Pregnancy-associated hypertension (PAH) affects upwards of 8% of all pregnant around the world. Disorders that are associated with abnormal blood pressure levels during pregnancy include preeclampsia (PE), eclampsia, pregnancy-induced hypertension, and chronic hypertension. PAH detrimentally impacts both the mother and the fetüs.

PE occurs at a frequency of 5-8% of all pregnancies [1]. Previous preeclampsia, nulliparity, age < 18 years or > 40 years, chronic hypertension, chronic kidney disease, family history of preeclampsia, and obesity are some well-known conventional risk factors for PE development [2, 3]. Preeclampsia is a serious concern of obstetrics because of the bearing of potential worse outcomes. PE-associated outcomes are; i) obstetric-related (intrauterine growth restriction, preterm delivery, abruption placenta, even maternal and fetal deaths), ii) long-term maternal risk (chronic hypertension, cardiovascular disease [CVD], death from CVD, stroke, etc) [4]. Although, there is no available standardized predictive laboratory test that could assist in diagnosing the disease, the utility of such a test like lipcalin-2 (LCN-2) would be valuable, at least in managing the obstetric course of pregnants with a higher risk for PE development.

It is assumed that abnormal and immature vascularization of the placenta and its products might have a key role in the pathogenesis of the disease. Consequently, impairment of trophoblastic invasion to the endometrium, endothelial cytokines, chemokines, and angiogenic factors (for example, soluble flt-1) all have key roles in the development of PE.

LCN-2 is a protein encoded in humans, secreted by neutrophils, and that serves as a mediator of innate immunity. LCN-2 is expressed in the kidney, prostate, respiratory system and gastrointestinal system where it restricts bacterial growth by sequestrating iron-containing siderophores [5-7]. It works similarly in the mammalian cell's cytoplasm by binding to the mammalian siderophore 2,5-dihydroxybenzoic acid (2,5-DHBA). This complex prevents excessive free iron accumulation in the cytoplasm. Mammalian cells lacking 2,5-DHBA accumulate abnormal intracellular levels of iron leading to high levels of reactive oxygen species and subsequent cellular injury [5, 6]. LCN-2 expression increases during inflammation, infection, ischemia, neoplastic invasion and neoplastic transformation [8]. It is resistant to proteases, so its levels increase during celluar injuries.

PE is primarily diagnosed on the basis of high blood pressure. The severity of the disease is linked to the sever-

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Table 1. — *Criteria for the diagnosis and classification of preeclampsia.*

Definition of Preeclampsia;
Systolic blood pressure >140 mmHg and/or diastolic blood pressure; 90 mmHg
*two occasions at least 4 hours apart after 20 weeks in previously non-hypertensive patients
** and one or more of following;
· Proteinuria: ≥ 0.3 g/day in a 24h urine collection or the equivalent
· Low level of platelets: < 100,000 /microL
· High serum creatinine: > 1.1 mg/dL or doubling of creatinine in the absence of any other causes of renal disease
· Elevated serum transaminases: at least two-fold rise
· Pulmonary edema
· Cerebral or visual symptoms
Severe Preeclampsia;
Systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 110 mmHg and proteinuria (with or without signs and symptoms of significant end-organ dysfunction).
or
Systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg (with or without proteinuria) and one or more of the mentioned signs and symptoms of significant end-organ dysfunction.

ity of the increase in blood pressure. Given the pathogenesis of the disease, it is reasonable to consider an acute inflammatory state during the disease. Indeed, LCN-2 levels have been investigated in many acute and chronic diseases. However, conflicting results have been reported in small sample-sized studies in regards to LCN-2 levels in PE [8, 9]. Therefore, its potential role in PE, including potential prognostic value, needs further elucidation.

Increased vascular resistance, endothelial damage, impaired vascular integrity, and problems associated with coagulation and platelet function all can contribute to uteroplacental insufficiency and consequently can lead to PE [10]. Therefore, uterine artery flow characteristics might be informative in predicting PE. Uterine artery waveforms are a better indicator than intrauterine growth restriction in predicting PE [11, 12]. Overall, a pulsatility index combined with notching can predict PE better [13].

In our study, we investigated the association between plasma LCN-2 levels, uterine artery flow characteristics by Doppler USG, and the presence and severity of PE.

Material and Methods

The study was conducted in Van Yuzuncu Yil University between October 2013 and May 2014. In total, there were 120 participants enrolled in the study: 51 pregnant women with severe PE, 27 with mild PE, and 42 with uncomplicated pregnancies (no PE). PE was defined and classified as mild and severe (Table 1) according to the recommendations of the "American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy, 2013" [14]. All individuals gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Van Yuzuncu Yil University; the university scientific research committee approval number: 018.05.02.2013.

The demographic features of all participants were noted. Gestational age was calculated according to the last menstrual period in regularly menstruating pregnant women and by using USG evaluation performed in the second or third trimester of pregnant women with irregular menstruation periods. Blood samples were drawn from all pregnant women, centrifuged, and then stored in a -20 freezer. Serum LCN-2 levels, hemograms, liver and kidney function tests, and spot urine protein/creatinine ratios were ascertained. A biotin-labeled antigen was adapted to a sandwich enzyme-linked immunosorbent assay (s-ELISA) for detection of LCN-2 (BioVendor Research and Diagnostic Products Human Lipocalin-2 Elisa Cat. No: RD191102200R, manufactured by BioVendor, LLC, 1463 NC, USA). Plasma LCN-2 levels were studied at time of study enrollment for pregnant women with PE and just prior to delivery in women with non-complicated pregnancies. Doppler USG evaluation was performed more than once in women with PE and once before delivery in women with non-complicated pregnancies. Women with non-complicated pregnancies were followed up for more than 8 weeks after delivery to exclude post-partum PE/eclampsia. Preeclamptic pregnant women also were followed up for more then 8 weeks after delivery, in order to exclude subsequent PE. Doppler USG evaluation was performed by a single experienced radiologist.

Doppler velocimetry was evaluated by a radiologist using a V6-2 curved-array volume transducer. The pulsatility index of both uterine arteries was measured and their mean was taken for analyses.

IUGR was defined according to Alexander curve references for fetal growth using gestational age at delivery and actual birth weight [15].

Statistical Analysis

The study's data were analyzed using statistical package program for social science (S.P.S.S. Inc., Chicago, IL). Distribution of the data was studied by using the Kolmogorov-Smirnov test. One-Way ANOVA and posthoc Scheffe

Table 2. — *Demographic and labarotory findings of each group.*

	Normal Pregnancy[a] N = 42	Mild Preeclampsia[b] N = 21	Severe Preeclampsia[c] N = 57	*p* value
Age (years)	25.9 ± 6.7	26.2 ± 5.5	26.7 ± 5.2	*a vs b*; $p > 0.05$ *b vs c*; $p > 0.05$ *a vs c*; $p > 0.05$
BMI (kg/m^2)	26.8 ± 3.5	28.1 ± 3.6	28.4 ± 3.8	*a vs b*; $p > 0.05$ *b vs c*; $p > 0.05$ *a vs c*; $p > 0.05$
Gestational age at the sampling time (week)	33.9 ± 1.6	34.5 ± 1.2	34.1 ± 2.1	*a vs b*; $p > 0.05$ *b vs c*; $p > 0.05$ *a vs c*; $p > 0.05$
Gestational age during the delivery (week)	39.1 ± 1.2	38.8 ± 1.4	35 ± 2.1	*a vs b*; $p < 0.05$ *b vs c*; $p < 0.001$ *a vs c*; $p < 0.001$
Birth Weight (g)	3315 ± 412	2410 ± 298	2190 ± 298	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.05$ *a vs c*; $p < 0.001$
Sistolyic blood pressure (mmHg)	126.2 ± 15.9	152.5 ± 7.1	165.9 ± 9.5	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.05$ *a vs c*; $p < 0.001$
Diastolyic blood pressure (mmHg)	61.6 ± 9.3	90.3 ± 5.5	101.6 ± 7.4	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.001$ *a vs c*; $p < 0.001$

tests for parametric data and Kruskal-Wallis test for non-parametric data were used, in order to compare the groups. $p < 0.05$ was considered significant between groups. Correlation between parameters was studied by Spearman's and Pearson's correlation tests.

Results

The demographic and laboratory data of participants are shown in table 2. The mean age of the women in the study was 25.6 ± 4.7. The average gestational age at which blood samples had been taken was similar between the groups. Gestational age at the time of delivery was higher in the uncomplicated pregnancy group compared to the preeclamptic groups ($p < 0.05$), but similar between the mild and severe preeclamptic groups ($p > 0.05$). Presence of the severity of PE correlated with IUGR.

The number of pregnant women with blood flow impairment as determined by Doppler ultrasound evaluation was higher in women with severe PE, and LCN-2 levels were positively correlated with the presence and the severity of PE (Table 3). Patients with severe PE also were involved in 20/24 of all HELLP cases.

Discussion

PE is characterized by newly diagnosed hypertension manifesting 20 weeks into a pregnancy. While clinical sequeale is variable, in severe cases, there can be multi-organ damage. Overall, PE is a common disease that causes both maternal and perinatal mortality and morbidity. Defining risk factors and achieving early diagnosis are both critical in the management of the disease. Serum uric acid, kreatinin levels, and proteinuria are some of the laboratory parameters which are useful in the diagnosis and prognosis of the disease [16]. In our study, we revealed that serum LCN-2 levels are high in PE and correlate with the severity of the disease. Therefore, LCN-2 can aid in diagnosing PE.

Abnormal placentation is the apparent consequence of pathophysiological changes in developing PE. During normal pregnancy, cytotrophoblasts emigrate from the chorionic villi and invade the uterus, reaching the inner third of the myometrium. Within the uterine wall, cytotrophoblasts deeply invade the spiral arteries. They also insert themselves among the smooth muscle cells that form the tunica media. As a result, the spiral arteries attain the physiologic properties that are required to perfuse the placenta adequately. In PE, on the other hand, cytotrophoblastic invasion of the interstitial uterine compartment is frequently shallow, although not consistently so. In many locations, spiral artery invasion is incomplete [17].

Overall, possible mechanisms offered to explain the pathophysiology behind PE are: i) abnormal remodeling of the spiral arteries, ii) abnormal differentiation of trophoblasts, and iii) placental hypoperfusion, hypoxia, and ischemia, resulting in abnormal placentation [17, 18]. Additionally, environmental factors, genetic factors, immunologic factors, inflammation, complementary system activation, and systemic endothelial damage likely have some key roles in the pathogenesis of PE [19]. For instance, soluble fms-like tyrosine kinase 1 (sFlt-1) is a natural antian-

Table 3. — *High levels of LCN-2 are associated with Doppler Blood flow abnormality, the presence of HELLP syndrome and IUGR.*

	Normal Pregnancy[a] N = 42	Mild Preeclampsia[b] N = 21	Severe Preeclampsia[c] N = 57	*p* value
LCN-2 (ng/μL)	129.3 $\pm$ 74.8	172.6 $\pm$ 82.8	197.9 $\pm$ 80	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.001$ *a vs c*; $p < 0.001$
Blood flow anbormality in Doppler (yes/no), n	1 (%14.1)	4 (%14.8)	12 (%23.5)	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.001$ *a vs c*; $p < 0.001$
HELLP (yes/no), n	1 (%2.4)	3 (%11.1)	20 (%39.2)	*a vs b*; $p < 0.001$ *b vs c*; $p < 0.001$ *a vs c*; $p < 0.001$
IUGR (yes/no), n	4 (%9.5)	7 (%25.9)	13 (%25.5)	*a vs b*; $p < 0.001$ *b vs c*; $p > 0.05$ *a vs c*; $p < 0.001$

giogenetic mediator that binds to angiogenic factors such as vascular endothelial growth factor and placental growth factor. In PE, sFlt-1 activity increases via excess placental secretion. As a result, sFlt-1 has been thought to have a key role in the pathogenesis of PE by reducing placental blood vessels growth [20-21]. Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL), is a protein involved in innate immunity, and functions by sequestrating iron from the cytoplasm. It is resistant to proteases and can be detected in urine, feces, and serum immediately after an inflammatory process [22].

Given the role of inflammation in the pathogenesis of PE [23], researchers also have evalulated LCN-2 levels in PE. Karampas *et al*. have reported that LCN-2 levels tend to rise in the course of pregnancy. However, consistent with our data, the magnitude of the increase in LCN-2 was significantly greater in preeclamptic pregnant women compare to non-preeclamptic women. Moreover, serum lipocalin levels were also found to be elevated in the first and second-trimester of pregnant women whom later developed PE [24, 25]. In our study, we also found LCN-2 levels are higher in severe PE compared to mild PE.

Our study design slightly differs than in previous studies. Because we are a tertiary and intensive obstetric unit in the city, we were capable of including many severe preeclamptic patients in the study. In women with uncomplicated pregnancies, LCN-2 levels were studied at the end of the third trimester and found lower than patients who had clinical and laboratory findings of PE. Follows-up continued 6-8 weeks after delivery and no PE cases were noted. In contrast to many studies, Arikan *et al*. found a low level of LCN-2 in PE [9]. It is reasonable to consider PE as a multifactorial disease and to understand that its pathogenesis may not be captured solely on the basis of one or more serum inflammatory biomarkers.

LCN-2 levels also have been studied in pregnant women who have risk factors for developing PE, in the prepregnancy period. Cesur *et al*. found LCN-2 levels were higher in cases whose prepregnancy body mass indexes were greater than 25 kg/m^2 [26].

Uteroplacental blood flow can be easily measured by using Doppler USG of the uterine arteries. This method can predict the level of risk for PE, and IUGR, since it is known the impeded blood flow in the uterine artery decreases with increasing gestational age [27]. In the evaluation of the uterine artery, many indices can be calculated and assessed. We used Pulses-wave Doppler USG in our study and found high uterine artery pulsatility indices in preeclamptic women. Multivariate analyses revealed a strong association between the higher LCN-2 levels and Doppler USG findings. Youssef *et al*. have reported that, when combined with PAPP-A, PIGF, sFlt-1, P-selectin and NGAL levels high uterine artery pulsatility index and associated uterine artery Doppler findings can predict mean risk for mild PE at 8.8%, and for severe PE at 38.6% [28].

Our study has some limitations. We evaluated LCN-2 levels only one time, and this was in the third trimester. We did not evaluate LCN-2 levels at other times, for instance, following delivery. Since we are a tertiary care unit, the majority of preeclamptic patients were referred to our clinic from various hospitals and therefore additional data from these patients were not available.

In conclusion, PE is a serious pregnancy-related complication that can result in poor outcomes of both the mother and the fetus. Early diagnosis allows for appropriate management of the disease to prevent complications. Our findings revealed that LCN-2 levels and Doppler USG findings provide additional benefits in the evaluation of PE. Overall, high LCN-2 levels may serve as in important prognostic factor in the development of PE.

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Conflict of Interest

The authors declare that no conflict of interest.

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