ORIGINAL ARTICLE

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The Effect of Serum Laminin Level on Obstetric Outcomes in Pregnants with Preeclampsia

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Abstract

Objective: To evaluate maternal serum laminin level in cases with preeclampsia, and to investigate the relationship of laminin with clinical parameters and perinatal outcomes in this case group.

Methods: In this prospective observational study, 62 pregnant women with preeclampsia and 76 healthy normotensive pregnant women, matched for maternal age and body mass index, were evaluated. Maternal serum laminin levels were measured by ELISA and compared between groups.

Results: Maternal serum laminin levels were significantly higher in the preeclampsia group (p=0.001). Laminin was significantly positively correlated with mean arterial pressure and amount of proteinuria (p=0.005, p=0.000; respectively), while significantly negatively correlated with umbilical cord pH and week of delivery (p=0.000, p=0.001; respectively). ROC curve analysis and Youden's index showed that the optimal threshold for laminin was 53.95 ng/mL when it comes to distinguishing pregnancies with preeclampsia from controls, with 65% sensitivity and 59% specificity.

Conclusion: In conclusion, this study showed that serum laminin levels in pregnant women with preeclampsia were significantly higher than in the healthy normotensive control group. It was also reported that laminin levels were positively correlated with mean arterial pressure and proteinuria. We think that these findings point to the role of laminin in the pathogenesis of preeclampsia.

Keywords: Preeclampsia, laminin, mean arterial pressure, proteinuria, pregnancy

INTRODUCTION

Pre-eclampsia is an important disease that develops in 2-8% of all pregnancies and progresses with maternal, fetal, and neonatal morbidity and mortality (1,2). The pathophysiology of pre-eclampsia has not yet been attributed to a definite cause so far. Inappropriate remodelling of spiral arteries, insufficient cytotrophoblastic invasion associated with uteroplacental hypoperfusion, and endothelial damage caused by antiangiogenic factors released from the is chemic placenta into the maternal circulation are thought to play a role in the pathophysiology of pre-eclampsia. Additionally, an increase in inflammatory cytokines is observed as a result of endothelial damage. Chronic uteroplacental ischemia, immune maladaptation, genetic factors, increased trophoblast apoptosis or necrosis, and an increased inflammatory response against trophoblasts play an important role in the pathophysiology of pre-eclampsia (1,2).

Laminins are a family of glycoproteins that comprise the basement membrane (3). Cell surfaces provide the integrity of the basement membrane by adhering to collagen type IV, heparan sulfate. Laminins trigger intracellular signals by interacting with cell surface receptors to regulate implantation and placentation (3,4). Laminin is required for trophoblast invasion of the uterine decidua and maternal vascular system and for successful embryo implantation (3,4).

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The placenta is an organ rich in the basement membrane (5,6). In some studies, it has been observed that serum laminin values increase during pregnancy with increasing placental volume, remain at high levels after reaching a plateau in the third trimester, and decrease with delivery (7). This relationship between the placenta and laminin may shed light on the pathogenesis of preeclampsia, which develops because of defective placentation.

The aim of our study is to evaluate maternal serum laminin levels in cases with preeclampsia and to investigate the relationship of laminin with clinical parameters and perinatal outcomes in this case group.

METHODS

Our study is a prospective observational study conducted between June 2021 and June 2022 at the University of Health Sciences Turkey Prof. Dr. Cemil Taşcıoğlu City Hospital, Obstetrics and Gynecology Clinic. This included 62 pregnant women with pre-eclampsia and 76 healthy normotensive pregnant women, matched for maternal age and body mass index (BMI), and who presented continuously to the clinic. Women younger than 18 years old and older than 45 years those with multiple pregnancies, presence of fetal anomaly, diabetes, renal or autoimmune disease were excluded from our study.

The diagnosis of preeclampsia was defined as hypertension (systolic and/or diastolic blood pressure of 140 and/or 90 mmHg measured at least 4 hours apart) and proteinuria (≥300 mg in 24 h urine or urine protein/creatinine ratio ≥0.3) beginning after 20 weeks of gestation in a previously normotensive woman. Cases with new-onset hypertension but not accompanied by proteinuria were included in the preeclampsia group if they had the following signs and symptoms, which show end organ tissue damage: headache unresponsive to medical therapy, visual impairment, pulmonary edema, platelet count $<100x10^9$/L, serum creatinine concentration more than 1.1 mg/dL or a doubling of the serum creatinine concentration in the absence of other renal disease, elevated blood concentrations of liver transaminases to twice normal concentration (1). The case group was divided into subgroups. During this process, pregnant women diagnosed with preeclampsia before 34 weeks of gestation were included in the early-onset preeclampsia (EOPE) group (n=35), whereas pregnant women diagnosed after 34 weeks of gestation were considered to have late-onset pre-eclampsia (LOPE) (n=27) (8).

Five milliliters of venous blood samples were collected from all participants after 12 h of fasting and placed in vacuum tubes without anticoagulant. These tubes were centrifuged at 2,000 rpm for 20 minutes, and the serum samples obtained were placed in an Eppendorf tube and frozen at -40 °C until analysis. The samples were brought to room temperature at the time of analysis. Collected serum samples were measured with Microplate Reader RT 2,100 C and Microplate Washer RT 2,600 C instruments with Human Laminin ELISA kits (Bioassay Technology Laboratory, Shanghai, China, catalog no. E4996Hu). Serum laminin values were measured in pg/mL and the reference range was determined as 6.5-400. This study was approved by the Medical Ethical Committee of the University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital (no: 236, E-48670771- 514.10; date: 21.06.2021). In our study, which was conducted in accordance with the Declaration of Helsinki, informed consent was obtained from all patients.

Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences software version 25.0 (Chicago, IL, USA). The Kolmogorov-Smirnov test was used to evaluate the normality of the distribution of the variables. Data were presented as mean ± standard deviation. The Mann-Whitney U test and Kruskal-Wallis test were used for statistical comparisons between groups, and Dunnett's T3 post-hoc test was preferred for multiple comparisons. Categorical variables were expressed as a percentage of the total. Pearson's chi-square independence test was used to examine the interdependence between categorical variables. Pearson correlation analysis was performed to evaluate the correlations between parametric variables. A receiver operating characteristic curve and Youden index were used to determine an optimal laminin cut-off point for the diagnosis of pre-eclampsia. $P<0.005$ value was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the study groups are shown in Table 1. There was no significant difference between the groups in terms of age, gravidity, parity, BMI, and blood collection time. Week of birth, birth weight, umbilical cord pH, and 5th minute APGAR score were found to be significantly lower in the preeclampsia group than in the control group ($p<0.001$, $p<0.001$, $p=0.021$, and $p=0.004$; respectively). Mean arterial pressure (MAP), neonatal intensive care unit requirement, and maternal serum laminin levels were higher in the pre-eclampsia group ($p<0.001$, $p<0.001$, $p=0.001$; respectively).

The comparison of the laminin levels of the pre-eclampsia subgroups is presented in Table 2, 3. Maternal serum laminin

levels were found to be significantly higher in the EOPE group than in the LOPE group (p=0.105).

When the correlation of maternal serum laminin levels with demographic and clinical parameters was examined, laminin showed a significant positive correlation with mean arterial pressure and amount of proteinuria (p=0.005, p<0.001; respectively) and a significant negative correlation with umbilical cord pH and week of delivery (p<0.001 and p=0.004; respectively).

Receiver operating characteristic curve analysis and Youden's index showed that the optimal cut-off of laminin was 53.95 ng/mL to distinguish pre-eclamptic pregnancies from controls, with 65% sensitivity and 59% specificity (area under the curve: 0.664; 95% confidence interval, 0.574-0.753, p=0.001) (Figure 1).

Table 1. Comparison of demographic and clinical features of the cases

	Preeclampsia group (n=62)	**Control group (n=76)**	**p**
Age	32.4±5.5	30.8±5.3	0.054
Gravidity	2.7±1.6	2.5±1.6	0.575
Parity	1.4±1.3	1.2±1.3	0.444
BMI (kg/m²)	31.9±5.2	30.8±4.7	0.174
MAP	111.9±9.3	81.8±5.5	**0.000**
Proteinuria	755.5±1610.4	-	-
Blood collection time	32.3±2.8	32.1±1.7	0.691
Birth week	33.9±5.7	38.3±1.8	**0.000**
Birth weight (g)	2397±993	3176±549	**0.000**
Cord pH	7.31±0.07	7.34±0.04	**0.021**
5' APGAR	8.42±1.4	8.87±0.5	**0.004**
NICU	31/62 (50%)	14/76 (18.4%)	**0.000**
Laminin (pg/mL)	83.9±72.2	50.9±20.1	**0.001**

BMI: Body mass index, MAP: Mean arterial pressure, NICU: Neonatal intensive care unit

Table 2. Comparison of laminin levels between preeclampsia subgroups and the control group

	Laminin (pg/mL)	**p**
EOPE group (n=35)	99.5±86.4	0.105
LOPE group (n=27)	63.8±41.5	**0.007**
Control group (n=76)	50.9±20.1	0.336

EOPE: Early onset pre-eclampsia, LOPE: Late onset pre-eclampsia

Table 3. Correlation of laminin with demographic and clinical parameters

	Laminin	
	R	**p**
Age	-0.089	0.296
BMI	-0.003	0.969
MAP	0.237	**0.005**
Proteinuria	0.566	**0.000**
Cord pH	-0.500	**0.000**
Week of birth	-0.241	**0.004**
Birth weight	0.060	0.481
5' APGAR	0.065	0.448

BMI: Body mass index, MAP: Mean arterial pressure, APGAR: Appearance, pulse, grimace, activity, respiratory

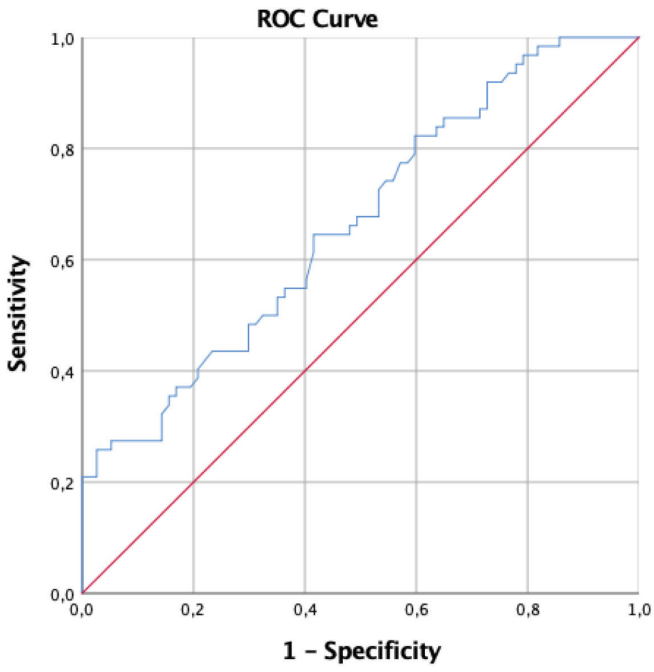

Figure 1. ROC curve for laminin levels

DISCUSSION

In our study, we planned to investigate maternal serum laminin values in pre-eclamptic pregnant women and to evaluate the relationship between laminin levels and laboratory parameters and perinatal outcomes in this case group. We found that maternal serum laminin levels were significantly higher in pregnant women with pre-eclampsia than in the control group in our study. Maternal serum laminin level was especially higher in cases with early-onset pre-eclampsia. In addition, while maternal serum laminin levels showed a significant positive correlation with MAP and the amount of proteinuria, it showed a significant negative correlation with cord pH and week of delivery.

Laminin plays an important role in cell proliferation, migration, and invasion for trophoblast cells and probably contributes to the development of pre-eclampsia by regulating the PI3K/Akt/mTOR signaling pathway in trophoblasts (9). Insufficient adhesion between extravillous trophoblasts and the extracellular matrix during placental formation is a basic element of the pathogenesis of early-onset pre-eclampsia (10,11). In late-onset preeclampsia, although placentation is normal, a widespread inflammatory response triggered by maternal microvascular diseases and hypoxia is observed (11,12). In our study, the reason for the higher maternal serum laminin values in cases with early-onset pre-eclampsia may be the initiation of the destruction of the laminin molecule during the placentation stage. In our study, patients with late-onset preeclampsia had higher serum laminin values than the control group, and we believe that this is related to the widespread systemic inflammatory response involved in the pathophysiology of late-onset pre-eclampsia. Koutroubakis et al. (13) reported that serum laminin levels were higher in the active period in individuals diagnosed with inflammatory bowel disease, in their study in which they compared the cases diagnosed with inflammatory bowel disease, another inflammation-based disease, and the healthy control group. This result is compatible with our study.

Furuhashi et al. (7), in their study in which they compared 19 healthy pregnant women, 21 pregnant women diagnosed with preeclampsia, and 22 healthy women who were not pregnant, found that serum laminin levels increased during pregnancy. This increase in laminin level has been attributed to the increased volume of the basal lamina-rich placenta during pregnancy. In the pre-eclamptic group, serum laminin levels were found to be significantly higher not only during pregnancy but also in the postpartum period compared to the healthy pregnant group (7). These findings point to changes and damage in the composition of the basement membrane. In addition, the same study argued that increased laminin levels may also occur due to glomerular damage, but stated that serum laminin levels did not correlate with the amount of proteinuria, urea, and creatinine values (7). Laminin is a glycoprotein that occupies an important place in the glomerular basement membrane structure, and changes in laminin level may affect villus permeability (14,15). In our study, there was a significant positive correlation between maternal serum laminin levels and proteinuria. The reason for this difference may be the higher number of cases in our study and the fact that we included cases with early-onset and severe pre-eclampsia.

Study Limitations

The main limitations of our study are that it was conducted in a single center and the relatively small sample size. Additionally, investigating laminin levels only in maternal serum and not investigating placental expression of the molecule is another important limitation. The strengths of our study are its prospective design, investigation of the laminin level in pre-eclampsia subgroups, and determination of an optimal threshold value for laminin in the prediction of the disease.

CONCLUSION

In conclusion, this study showed that serum laminin levels in pregnant women with pre-eclampsia were significantly higher

than in the healthy normotensive control group. It was also reported that laminin levels were positively correlated with MAP and proteinuria. We believe that these findings point to the role of laminin in the pathogenesis of pre-eclampsia.

Ethics

Ethics Committee Approval: This study was approved by the Medical Ethical Committee of the University of Health Sciences Turkey, Prof. Dr. Cemil Taşcıoğlu City Hospital (no: 236, E-48670771- 514.10; date: 21.06.2021).

Informed Consent: Consent was received from the patients who participated in this study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.Ö., N.Ç.Ç., S.G., Concept: H.Ö.Ç., B.Ç., V.M., Design: H.Ö., M.Ö., B.Ç., V.M., Data Collection or Processing: H.Ö., N.Ç.Ç., S.G., Analysis or Interpretation: H.Ö., N.Ç.Ç., M.Ö., Literature Search: H.Ö., S.G., V.M., Writing: H.Ö.Ç., M.Ö., B.Ç.

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