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Evaluation of serum perlecan levels in pregnancy with mild and severe preeclampsia

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ABSTRACT

Aim: To determine the levels of perlecan contributing to angiogenesis and autophagy inhibition in severe and mild preeclamptic women.

Material and Method: The study included a total of 89 patients as 3 group including severe preeclampsia as group 1 (n:30), mild preeclampsia as group 2 (n:30), and control as group 3 (n:29). All the groups were evaluated in terms of prepartum maternal serum perlecan levels.

Results: The Perlecan level of the group 1 was at higher levels than others ($p<0.0001$). The Perlecan level in the group 2 was higher than the control ($p<0.0001$). In the correlation analysis, AST ($p<0.0001$), ALT ($p<0.0001$), systolic blood pressure ($p<0.0001$), diastolic blood pressure ($p<0.0001$), creatinine ($p<0.0001$), proteinuria ($p<0.0001$), and LDH ($p<0.0001$) were positively correlated with perlecan level in the severe preeclampsia.

Conclusion: Serum perlecan levels were higher in preeclamptic pregnant women and this increased more especially in those with severe preeclampsia clinic.

Keywords: Perlecan, preeclampsia, autophagy, angiogenesis, pathophysiology

INTRODUCTION

Preeclampsia, characterized by hypertension and proteinuria, is one of the most important clinical conditions during pregnancy (1). It is seen in 3-5% of all pregnancies worldwide and seriously affects the life of the mother and baby (2).

Various factors have been suggested in studies investigating the conditions that prevent the development of the placental bed (3). There are crucial factors in the pathogenesis of preeclampsia such as systemic inflammation, hormonal and biochemical changes in the placenta, increase and decrease of proteins in maternal blood (4). Uteroplacental circulatory failure that develops as a result of placental dysfunction in the first trimester plays a critical role in its pathogenesis (5). Placental dysfunction reduces the invasion of trophoblasts into the decidua, and this decrease causes maternal hyperdynamic circulation and endothelial activation by causing villus hyperplasia, placental hypoperfusion, and vasopressor release by causing modification in spiral arteries (6,7).

Hypoxia occurring at the physiological level causes trophoblast invasion in the early period of placenta formation by affecting autophagy at the basal level (8). Autophagy reduces the aggregation of proteins in cells, that is, it has a protective effect on the cell; by inhibiting autophagy, proteins such as the amyloid increase in the cell and impair cell function (9). The findings explaining the relationship between preeclampsia and autophagy are still controversial.

In that term, Perlecan, a derivative of heparan sulfate, one of the main components of the basement membrane, comes to the fore because it increased angiogenesis and reduced autophagy according to the recent studies (10-12). Furthermore, perlecan level increases from the 17th week to the 40th week of pregnancy, but although its physiological role in pregnancy is not known exactly (13).

The present study aimed to analyze preaclampsia in separate groups and measured the prepartum perlecan levels in maternal serum to investigate its effect in preeclampsia.

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MATERIAL AND METHOD

Study Design

The study was designed as a level-II prospective observational clinical research and performed after the approval by Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University, Faculty of Medicine (Date: 29/08/2018, Decision No: 06, Session: 2018/5). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The written informed consents were obtained from the patients.

The study included a total of 89 patients who admitted to the Gynecology and Obstetrics Department on 2019 to 2020 as 3 group including severe preeclampsia as group 1 (n:30), mild preeclampsia as group 2 (n:30), and control as group 3 (n:29). All the groups were evaluated in terms of prepartum maternal serum perlecan levels.

Inclusion/Exclusion Criteria

Patients with 20 to 36 pregnancy weeks were included in the study. Patients with blood pressure above 140/90 mmHg and with proteinuria more than 300 mg/l in 24-hour urine were included in the mild preeclampsia group. In addition, those with blood pressure above 160/110 mmHg, those with more than twice as high AST and ALT values, those with creatinine value above 1.1 mg/dl, those with thrombocyte count less than 100,000 µl, those with pulmonary edema, those with epigastric pain and with no other diagnosis, those with a new-onset headache non-responsive to drugs, and those with visual symptoms were included in the severe preeclampsia group. Patients whose gestational week were compatible with the patients in the other group and who did not have chronic hypertension, gestational hypertension, gestational diabetes mellitus, renal disease, autoimmune disease, and multiple pregnancies were included in the control group.

Definitions of Preeclampsia

Mild preeclampsia: New onset blood pressure is 140 to 159 mmHg systolic and/or 90 to 109 mmHg diastolic. Proteinuria is 300 mg/24 hours; or ≥1+ on 2 random urine samples, collected at least 4 hours apart or protein: creatinine ratio is ≥0.3 mg/dL. In the absence of proteinuria, the following factors should be present: thrombocytopenia with platelets count <100,000/uL; serum creatinine ≥1.1 mg/L or a doubling of the serum creatinine concentration in the absence of another renal disease; impaired liver function with elevated blood concentrations of liver transaminases to twice normal concentration. In addition to them; Pulmonary oedema, Cerebral or visual disturbances,

Severe preeclampsia: BP is ≥160 mmHg systolic and/or ≥110 mmHg diastolic on two occasions and taken at least six hours apart or Proteinuria is 300 mg/24 hours; or ≥1+ on 2 random urine samples, collected at least 4 hours apart or protein: creatinine ratio is ≥0.3 mg/dL. In the absence of proteinuria, the following factors should be present: thrombocytopenia with platelets count <100000/ul, serum creatinine ≥1.1 mg/l or a doubling of the serum creatinine levels in the absence of another renal disease, the impaired liver function as indicated by elevated blood levels of liver transaminases to twice the normal concentration, pulmonary oedema, cerebral or visual disturbances.

Clinical Measurements

The gestational ages of the patients at the time of diagnosis were confirmed by the first-trimester crown-rump length and the last menstrual period. Gravida and the parity of all patients included in the study were questioned. Body mass indexes were calculated through height and weight measurements (kg/m^2). Their systolic and diastolic blood pressure values were recorded.

Laboratory Analysis

Proteinuria values were determined in 24-hour urine. Hemogram, AST, ALT, creatinine, uric acid, and LDH were tested. In addition, venous blood samples were taken from the antecubital area by phlebotomy method to simultaneously see the perlecan level of patients. The blood sample tubes were kept in an upright position for 10-20 minutes for coagulation and then centrifuged at +4°C and 4000 rpm for 10 minutes. Serum samples obtained were aliquoted and placed in the deep freezer at -80°C and kept until the day of analysis. On the day, when all serum samples were at room temperature, measurements were made using the enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. (BOSTER antibody and ELISA experts, Human Endorepellin/HSP2 ELISA Kit PicoKineTM Catalog number: EK1760, Boster Biological Technology, USA). Detection range of the kit was 0.624-40 ng/mL and detection sensitivity was <0.05 ng/mL.

Statistical Assessments

The collected patient data were analyzed using the Statistical Package For Social Sciences - SPSS for Windows 23.0 package (IBM Statistical Package, New York, USA). Mean and standard deviation were given as descriptive values for continuous data and variables with normal distribution, and the median for non-normally distributed variables. For comparisons between groups, the "ANOVA Test" was used for more than two groups in normally distributed variables, and the "Kruskal Wallis

H-Test" was used for more than two groups in non-normally distributed variables. Post-Hoc Tukey test was used to determine which parameters the significance arose from in the evaluations that were found to be significant. the possible relation between labaratory parameters and serum perlecan level was investigated with spearman correlation test. Results were considered statistically significant in cases where the p-value was less than 0.05.

RESULTS

There was no significant difference between the three groups in terms of age, body mass index (BMI), gravida, and parity between the groups. All three groups were evaluated in terms of perlecan levels. Group 1's perlecan level was found higher than the other groups and this difference was statistically significant when compared with the other two groups. Compared with the control group, a significant increase was found in favor of Group 2. There was also a significant difference between the groups in terms of SBP and DBP. There was no significant difference between the groups in terms of hemoglobin and platelet values (p=0.555, p=0.056, respectively). While there was no significant difference in AST and ALT values between group 2 and group 3, a significantly higher difference was found between group 1 and the other groups. While there was no significant difference between group 1 and group 2 in terms of creatinine values, a significant difference was observed between these groups and the control group. While there was no difference between group 1 and group 2 in terms of LDH and uric acid values, a significant difference was detected between these groups and the control group. In terms of proteinuria, significant differences were found between group 1 and group 2, between group 1 and the control group, and between group 2 and the control group (**Table 1**). In terms of correlation of perlecan levels in groups with hematological parameters, it was observed that perlecan level was positively correlated with SBP, DBP, AST, ALT, creatinine, proteinuria, and LDH levels in Group 1. It was observed that perlecan levels of Group 2 were positively correlated with AST, ALT, creatinine, proteinuria, LDH, and uric acid levels and negatively correlated with platelet levels (**Table 2**). The distribution and mean value of perlecan levels among the groups were found to be 2.43 ng/mL in the severe preeclampsia group, 1.22 ng/mL in the mild preeclampsia group, and 0.89 ng/mL in the control group. The distribution range of perlecan level was very wide in the severe preeclampsia group, while it was narrow in the control group (**Figure**).

Table 2. Analysis results of the correlation of perlecan levels of groups with other hematological parameters

Characteristics	Severe preeclampsia		Mild preeclampsia		Control	
	R	p values	R	p value	R	p value
SBP	0.506	*0.004*	0.322	0.082	0.087	0.653
DBP	0.594	*0.001*	0.321	0.083	0.107	0.580
Hb	0.104	0.585	0.169	0.373	0.352	0.061
Platelet	-0.286	0.125	-0.414	*0.023*	0.345	0.067
AST	0.755	*<0.0001*	0.429	*0.018*	0.326	0.084
ALT	0.697	*<0.0001*	0.423	*0.020*	0.285	0.134
Creatinine	0.554	*0.001*	0.412	*0.024*	0.171	0.376
Proteinuria	0.457	*0.011*	0.478	*0.008*	-0.242	0.205
LDH	0.619	*<0.0001*	0.385	*0.035*	0.176	0.361
Uric Acid	0.312	0.093	0.386	*0.035*	0.233	0.224

s: Spearman correlation analysis. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, Hb: Hemoglobin

Table 1. Distribution of demographic and clinical findings by groups

Characteristics	Total (n:89)	Severe Preeclampsia (Group 1) (n=30)	Mild Preeclampsia (Group 2) (n=30)	Control (n=29)	p-value
Age (year)	27 (15)	27 (15)	27 (13)	25 (14)	0.547
BMI (kg/m^2)	23.9 (20.2)	26.2 (20.2)	23.9 (16.2)	23.7 (18)	0.655
Gestational age (weeks)	32 (13)	33 (11)	31 (12)	32 (10)	0.188
Gravidity	2 (5)	2 (4)	2 (5)	2 (5)	0.995
Parity	1 (4)	0.5 (4)	1 (4)	1 (4)	0.997
Perlecan level (ng/ml)	1.22 (5.97)	2.43 (5.56)	1.22 (3.07)	0.89 (1.24)	*<0.0001*^{k}
SBP (mmHg)	145 (176)	164.5 (136)	148.5 (18)	113 (45)	*<0.0001*^{k}
DBP (mmHg)	92.28±18.4	107.5±17.4	95.36±7.1	73.27±7.95	*<0.0001*^{a}
Hb (gr/dl)	10.75±1.34	10.91±1.48	10.54±1.4	10.8±1.1	0.555
Platelet (10^3/μL)	214.3±67.5	194.5±69.7	212.7±68.7	236.4±58.9	0.056
AST (U/L)	19 (256)	29 (254)	19 (27)	18 (19)	*<0.0001*^{k}
ALT (U/L)	12 (258)	25.5 (257)	8.5 (27)	11 (25)	*<0.0001*^{k}
Creatinine (mg/dL)	0.53 (1.06)	0.65 (0.99)	0.54 (0.62)	0.42 (0.41)	*<0.0001*^{k}
Proteinuria (gr/day)	566 (4997)	1057 (4630)	709.5 (2808)	93 (159)	*<0.0001*^{k}
LDH (U/L)	214 (508)	273.5 (507)	255 (364)	144 (150)	*<0.0001*^{k}
Uric acid (mg/dl)	4.88±2.44	6.06±2.8	4.93±2.1	3.62±1.68	*<0.0001*^{a}

All the data were given as Mean±SD or Median (Clearance). a: Anova test, k: Kruskal Wallis test. BMI: Body mass index, Hb: Hemoglobin, SBP: Systolic blood pressure, DBP: Diastolic blood pressure

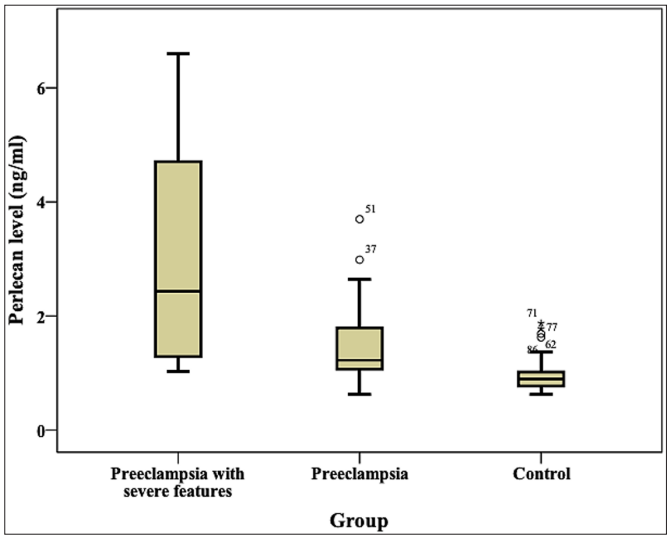

Figure. Distribution of perlecan levels between groups and their mean values

DISCUSSION

In our study, we found that the perlecan level was higher in the severe and mild preeclampsia group than in the control group. In addition, we found that perlecan level increased in the severe preeclampsia group in proportion to systolic and diastolic blood pressures, liver and kidney function tests.

In the study of Akbaş et al. (14) another study similar to our study, perlecan level was found to be high in severe preeclampsia cases. This study is important as it is a preliminary study that suggests that perlecan may play a role in the pathogenesis of preeclampsia. However, that study differs from our study in that the perlecan level was found to be high only in severe preeclampsia cases and a significant increase was not found in mild preeclampsia cases. In this study, we considered that perlecan level may be different in cases of preeclampsia and this may be due to the effects of proteoglycans on placental development and regulation of autophagy. The high perlecan level in both mild and severe preeclampsia cases we observed in our study supports the idea that perlecan may play a role in the pathogenesis of preeclampsia. In addition, the increased perlecan level in the severe preeclampsia group in proportion to systolic and diastolic blood pressures and liver and kidney function tests suggests that there may be a relationship between preeclampsia severity and perlecan level. It is possible to consider that as the severity of preeclampsia increases, the severity of autophagy, which is shown to be responsible for the pathogenesis process, also increases and the level of perlecan increases as a defense mechanism that prevents it.

Although Szenasi et al. (15) found increased perlecan levels in early preeclampsia in a similar study, they did not detect an increase in perlecan levels in late preeclampsia. In this study, it was concluded that the pathology of early preeclampsia was caused by abnormal trophoblast invasion-ischemic damage-placental stress, whereas late preeclampsia was based on a different pathology. In this study, it was found that the perlecan level decreased as the gestation period progressed. Yang et al. (16) found that perlecan level decreased as gestational age progressed, and perlecan level increased as gestational age progressed in those with gestational diabetes. In this study, hyperglycemia increased the level of perlecan. Confirming the information obtained from these studies, the high level of perlecan in preeclampsia suggests that perlecan is secreted as a defense molecule against abnormal processes in the body. It is a protective molecule and its level increases as a response to the abnormal autophagic reaction known to play a role in the pathogenesis of preeclampsia.

In another study for preeclampsia cases, it has been found that mRNA and protein expression of perlecan was significantly reduced. Perlecan has an angiogenesis-inducing effect and a decrease in perlecan level contributes to the pathogenesis of preeclampsia by reducing trophoblast invasion (17). Another study conducted to determine biological markers in cases with premature rupture of membranes found that perlecan level did not decrease in amniotic fluid, and although the role of heparan sulfate-derived proteoglycans in amniotic fluid is not known precisely, it has been considered to have a growth factor-like effect (13).

In a study by Zhao et al. (18) they showed that inhibition of protein kinase C beta increases autophagic activity and this increase triggers preeclampsia in both humans and animals. Recent studies report that the same inhibition triggers autophagy in preeclampsia after it has been shown that histone deacetylase inhibition triggers autophagy in multiple myeloma (19). Other studies report that autophagy activation is also associated with the enhancement of immune response and this affects the pathogenesis of preeclampsia (20). Similarly, we found the increased level of perlecan , although no evidence had about autophagy in our study. When the literature is reviewed, despite the findings of previous studies that inhibition of autophagy triggers preeclampsia, recent studies suggest that the increase in autophagy is more effective in the pathogenesis of preeclampsia (4).

A small number of patients and the lack of shorter gestational weeks are the limiting factors of our study. Besides, we should mention absence of placental level evaluation, absence of the parturition data, absence of umblical cord blood level and absence of the relation between perlecan level and neonate birthweight and APGAR score in the present study.

CONCLUSION

The maternal serum perlecan level was higher in the severe and mild preeclampsia group than in the control. In addition, perlecan level increased in the severe preeclampsia group in proportion to systolic and diastolic blood pressures, liver and kidney function tests. Based on the findings we obtained from our study consider that perlecan may be biochemical for understanding preeclampsia cases. Findings of the present study will be a guide for further studies to explain the relationship between perlecan and preeclampsia.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval was obtained from Clinical Research Ethics Committee of Kahramanmaraş Sütçü İmam University, Faculty of Medicine (Date: 29/08/2018, Decision No: 06, Session: 2018/5).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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