

ORIGINAL ARTICLE

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

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Abstract

In this study, we aimed to determine the levels of perlecan contributing to autophagy inhibition and angiogenesis in severe and mild preeclamptic women. A total of 89 patients including severe preeclampsia, group 1 (n:30), mild preeclampsia, group 2 (n:30) and control group, group 3 (n:29) were included in the study. All three groups were evaluated in terms of perlecan levels. Perlecan level in group 1 was determined at higher levels than other groups, and this level difference was statistically significant compared to the other two groups ($p<0.0001$). Group 2 was found to be significantly higher in favor of group 2 when compared with the control group ($p<0.0001$). Considering the distribution and average values of the perlecan level among the groups, 2.43 ng/ml in group 1, 1.22 ng/ml in group 2 and 0.89 ng/ml in the control group were detected. In our study, we found that 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 that occurs during pregnancy, and is called pregnancy disease [1]. It can be seen in 3-5% of all pregnancies worldwide and seriously affects the life of the mother and baby [2].

It is considered that uteroplacental circulatory failure that develops as a result of placental dysfunction in the first trimester plays a critical role in the pathogenesis [3]. In recent studies, it has been found that autophagy plays a role in the pathogenesis of preeclampsia. The fact that the findings that explain the relationship between preeclampsia and autophagy are still controversial, suggests that the studies on this subject will clarify the literature information.

Perlecan, which has been shown to increase angiogenesis and reduce autophagy in recent studies, is a molecule that has started to attract attention [4,5]. It has been shown that perlecan is a

derivative of heparan sulfate, one of the main components of the basement membrane, affects growth factors such as vascular endothelial growth factor and fibroblast growth factor, and plays a role in tumor growth and angiogenesis [6]. Furthermore, it is considered that the perlecan level increases from the 17th week to the 40th week of pregnancy, but although its physiological role in pregnancy is not known exactly, it has an effect like growth factor and plays an important role in the pregnancy process by increasing trophoblast invasion [7].

Studies to explain the effect of autophagy on the pathogenesis of preeclampsia have increased in the literature. Our aim in this study is to determine the level of perlecan, which is known to increase angiogenesis and prevent autophagy, in mild and severe preeclampsia cases, to investigate its effect in preeclampsia and to contribute to the literature on this subject.

Materials and Methods

This study was designed as a prospective observational study. The study was initiated after the approval of the Local Ethics Committee (Ethics Committee Decision No: 06, date: 29/08/2018, session: 2018/5). A total of 89 patients, including patients with severe preeclampsia (group 1, n=30), mild preeclampsia (group 2, n=30) and the control group (group 3, n=29) patients, were included in the

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study, who admitted to the Gynecology and Obstetrics Department on 01/01/2019 to 01/01/2020. Written informed consents of the patients included in the study were obtained according to the Helsinki declaration. 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 μ l, 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 pregnancy were included in the control group. 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 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. 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 $+4^\circ\text{C}$ 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, in accordance with the manufacturer's instructions. (BOSTER antibody and ELISA experts, Human Endorepellin/HSP2 ELISA Kit PicoKine™ 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.

The collected patient data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows 23.0 package program. 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. "Spearman Correlation Test" was used to show the relationship between perlecan levels and hematological variables between the groups. 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 1).

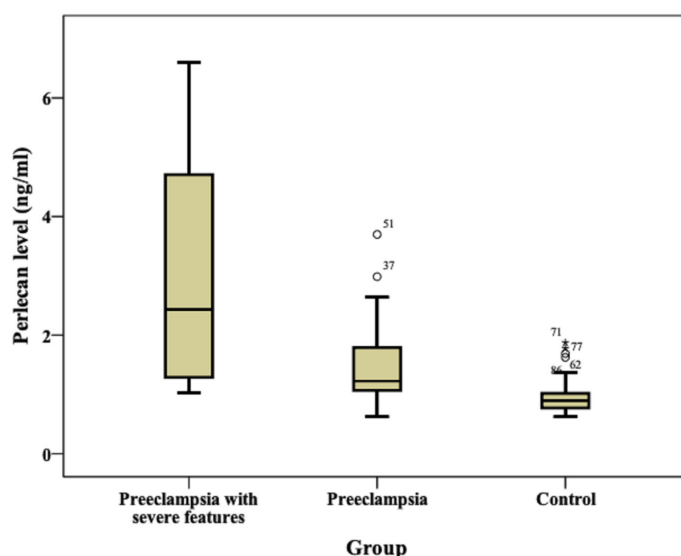


Figure 1. Distribution of Perlecan levels between groups and their mean value

Discussion

In our study, we found that the perlecan level was higher in the severe and mild preeclampsia group than 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.

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
	Mean ± SD or Median (Clearance)	Mean ± SD or Median (Clearance)	Mean ± SD or Median (Clearance)	Mean ± SD or Median (Clearance)	
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	107.56±17.42	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)	214.33±67.51	194.56±69.71	212.76±68.71	236.41±58.96	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

a: Anova Test, k Kruskal Wallis Test. (BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, Hb: Hemoglobin)

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)

In the study of Akbas et al., study similar to our study, perlecan level was found to be high in severe preeclampsia cases [8]. 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. 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 in the pathogenesis

process, also increases and the level of perlecan increases as a defense mechanism that prevents it.

Although Szenasi et al. found increased perlecan level in early preeclampsia in a similar study, they did not detect an increase in perlecan level 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 [9]. Yang et al. 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, we concluded that hyperglycemia increases the level of perlecan [10]. Confirming the information obtained from these studies, the high level of perlecan in preeclampsia

cases observed in our study suggests that perlecan is secreted as a defense molecule against abnormal processes in the body and that 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. We concluded that perlecan has an angiogenesis inducing effect and that a decrease in perlecan level contributes to the pathogenesis of preeclampsia by reducing trophoblast invasion [11]. This study is important in terms of showing its effect on angiogenesis, and the fact that we detected an increased level of perlecan in our study suggests that the increase in perlecan also has a protective effect from preeclampsia by inducing angiogenesis. 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 [7]. The increase in perlecan level in this study also suggests that perlecan has a protective effect during pregnancy and is a physiologically important molecule in terms of the continuity of the physiological functions of pregnancy.

In a study by Zhao et al., they showed that inhibition of protein kinase C beta increases autophagic activity and this increase triggers preeclampsia in both humans and animals [12]. 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 [13]. Other studies report that autophagy activation is also associated with the enhancement of immune response and this has an effect on the pathogenesis of preeclampsia [14]. The increased level of perlecan we found in our study suggests that perlecan increased secretion to inhibit the autophagy that occurs in preeclampsia. When the literature is reviewed, despite the findings of previous studies that inhibition of autophagy triggers preeclampsia, recent studies and our findings suggest that the increase in autophagy is more effective in the pathogenesis of preeclampsia [15].

A small number of patients and the lack of shorter gestational week are the limiting factors of our study.

Conclusion

Based on the findings we obtained from our study and the information we obtained from the literature, we consider that perlecan may be a biochemical biomarker in preeclampsia cases due to its effects on angiogenesis and autophagy. We think that perlecan is an important marker that can be used to diagnose

preeclampsia in the clinic and to distinguish between severe and mild preeclampsia. In addition, we think that looking at the perlecan level early in pregnancy can be useful in predicting preeclampsia. However, comprehensive studies should be conducted to support the findings on this subject.

Conflict of interests

The authors declare that they have no competing interests.

Financial Disclosure

All authors declare no financial support.

Ethical approval

The ethics committee decision was obtained from the Ethics Committee of Kahramanmaraş Sutcu Imam University with Session No. 2018/5, Decision No. 06 on 29.08.2018.

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