J Surg Med. 2022;6(9):820-823.
DOI: 10.28982/josam.7429

Research article

Journal of Surgery and Medicine
e-ISSN: 2602-2079

Exploring the relationship between preeclampsia and human epididymis protein 4

Gamze Nur Cimilli Senocak [1], Bunyamin Borekci [1], Zekai Halici [2], Emsal Pinar Topdagi Yilmaz [1]

[1] Department of Obstetrics and Gynecology, Ataturk University, Erzurum, Turkey
[2] Department of Pharmacology, Ataturk University, Erzurum, Turkey

ORCID ID of the author(s)

GNCS: 0000-0002-6750-9210
BB: 0000-0002-5917-1643
ZH: 0000-0001-6854-6059
EPTY: 0000-0001-8593-5726

Corresponding Author
Gamze Nur Cimilli Senocak
Ataturk University, Department of Obstetrics and Gynecology, Erzurum, Turkey
E-mail: gncimilli@gmail.com

Ethics Committee Approval
Ethics Committee approval was taken from the Atatürk University Clinical Research Ethics Committee, May 30, 2019 (numbered: B.30.2.ATA.0.01.00/251).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
The authors declared that this study has received no financial support.

Published
2022 September 26

Abstract

Background/Aim: The level of human epididymis protein 4 (HE4), a glycoprotein and protease inhibitor, increases under many malignancies and inflammatory conditions. HE4 is also associated with cell invasion, migration, and adhesion. In this study, we compared the HE4 protein levels in pregnant patients with preeclampsia to healthy pregnant and non-pregnant individuals with the aim of finding a biomarker that can be used to recognize preeclampsia.
Methods: Our study is a prospective case control study and included 20 pregnant women with preeclampsia, 20 pregnant women without preeclampsia, and 20 healthy non-pregnant women (the control). The participants' serum HE4 levels were analyzed statistically.
Results: Data analysis revealed that the mean HE4 levels were significantly lower in the preeclampsia group than in the other two groups (P = 0.002). Mean HE4 protein levels were also lower in the non-pregnant women than in the pregnant women without preeclampsia; however, this difference was not significant.
Conclusion: It is difficult to predict preeclampsia, and there is not any sensitive or specific biomarker for determining the condition. This study may support that HE4 protein may be useful and significant in predicting preeclampsia. The results we achieved provide proof that HE4 levels could be a potential biomarker for preeclampsia. Many more comprehensive studies are needed to support the association between HE4 protein and preeclampsia.

Keywords: HE4 protein, Preeclampsia, Protease inhibitor

How to cite: Cimilli Senocak NG, Borekci B, Halici Z, Topdagi Yilmaz EP. Exploring the relationship between preeclampsia and human epididymis protein 4. J Surg Med. 2022;6(9):820-823.

Introduction

Preeclampsia is one of the leading causes of morbidity and mortality during pregnancy and the postpartum period [1] and develops in approximately 4.6 % of pregnancies worldwide [2]. As the pathogenesis and etiology of preeclampsia are not yet fully understood, it is not always possible to predict [1, 3]. Although several mechanisms have been proposed to explain the development of preeclampsia, one of the most commonly accepted theories for its pathogenesis is abnormal placental formation due to defective cytotrophoblast invasion [3, 4]. During normal placental formation, cytotrophoblasts invade the uterus and the muscular layer of the spiral arteries [3]. Investigations of the etiopathogenesis of preeclampsia have primarily revealed the pathology of impaired cytotrophoblast invasion and defects in the molecules that facilitate this invasion, including surface adhesion molecules, such as integrins and molecules that aid invasion, such as metalloproteinases [4]. In particular, metalloproteinases facilitate the movement of cytotrophoblasts across the basement membrane; these cytotrophoblasts then invade the muscular layers of the uterine wall and spiral arteries—like a cancerous invasion—which is one of the most important stages of placental formation. Experimental studies have shown that various matrix metalloproteinase inhibitors disrupt cytotrophoblast invasion [5].

Human epididymis protein 4 (HE4) is a protease inhibitor, first isolated from human epididymal epithelial cells, which is known to be associated with many diseases [6]. Several studies have been conducted to determine the role of HE4 in gynecological malignancies, such as ovarian and endometrial cancers, or in benign gynecological diseases, such as endometriosis, which are known to progress upon organ invasion [6-8]. HE4 is also associated with several organ malignancies, such as breast adenocarcinomas, pulmonary cancers, and mesotheliomas [8, 9].

HE4 is actually a whey acidic protein (WAP), and studies have shown that it acts as a protease inhibitor, like other WAPs. It is also known to play a role in regulating cell migration, adhesion, and invasion [8, 10, 11]. Thus, HE4 may be responsible for impaired cytotrophoblast invasion during the development of preeclampsia. Moreover, because it is a protease inhibitor, it may cause impaired cytotrophoblast invasion (an important mechanism in the development of preeclampsia) by inhibiting the metalloproteinases needed for the process.

Studies have shown that cancer antigen (CA) 125 values increase during pregnancy and in cases of preeclampsia [12]. Considering that HE4 has been associated with many diseases, such as cancer and endometriosis, diseases that are also associated with increased CA 125 levels, it is necessary to investigate the relationship between preeclampsia and HE4 [6-8]. Thus, we investigated HE4 levels in patients with preeclampsia with the aim of identifying a new biomarker to predict the disorder.

Materials and methods

Study design

This study was designed as a prospective case control study. Three groups were involved in the study: the preeclampsia group, the healthy pregnant group, and the control group. Inclusion criteria for the preeclampsia group entailed having a gestational age >34 weeks, no chronic systemic disease, a preeclampsia diagnosis during this pregnancy period, and being between 18-45 years of age. Inclusion criteria for the healthy pregnant group entailed having a gestational age >34 weeks, no chronic systemic disease, no preeclampsia diagnosis during this pregnancy period, no pregnancy problems, and being between 18-45 years of age. Inclusion criteria for the control group entailed being between 18-45 ages, no chronic systemic disease, and not being pregnant.

According to our power analysis ($\alpha = 0.05$; 1-β [power] = 0.080), we required at least 20 participants for each group sample. An appropriate sample size, given the population size and specified combination of precision, confidence, and variability, was 45.

This study included 20 preeclamptic pregnant women with gestational ages of >34 weeks (preeclampsia group), 20 healthy pregnant women with gestational ages of >34 weeks (healthy pregnant group), and a control group comprising 20 healthy non-pregnant women (non-pregnant group). All visited the outpatient clinic of our university hospital between January 1, 2020, and January 1, 2021 and all participants were included in the study after obtaining their verbal and written informed consent. The study was initiated following the approval of Atatürk University Clinical Research Ethics Committee (numbered: B.30.2.ATA.0.01.00/251). The preeclampsia diagnosis was determined according to the criteria set by the American College of Obstetricians and Gynecologists (ACOG): new-onset hypertension occurring after 20 weeks of gestation or in the postpartum period in a woman with previously normal blood pressure (systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg in the left lateral decubitus position measured at least twice and at least four hours apart) that is accompanied by proteinuria and/or end-organ damage [13]. Proteinuria is defined as having protein levels > 300 mg/L in 24-hour urine samples. Additionally, the ACOG has defined preeclampsia in patients without proteinuria as the presence of elevated blood pressure with at least one of the following disorders: renal failure (serum creatinine levels of > 1,0 mg/dL or doubling of creatinine concentration), liver involvement (liver transaminase levels of > 40 IU/L), pain in the right upper quadrant of the abdomen, neurological complications (eclampsia, stroke, visual scotoma, and/or severe headaches), hematological complications (thrombocytopenia with ≤ 100,000 platelets/mm^3), and pulmonary edema [13].

Patients who had conditions that met the definition of preeclampsia (e.g., multiple pregnancies, fetal anomalies, suspected hepatitis A, B, or C or other infectious hepatitis, or renal disease before or during pregnancy) or who had gestational hypertension, chorioamnionitis, premature rupture of membranes, diabetes mellitus, chronic hypertension, a multisystem disease, hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, or those who smoked were excluded from the study. Pregnant women without any medical or obstetric pathology before or during pregnancy were included in the healthy pregnant women group. The non-pregnant group

consisted of healthy women who had visited the outpatient clinic for routine gynecological examinations.

Serum samples and laboratory assays

Venous blood samples were collected to measure the HE4 protein levels. After centrifugation, the blood serum was separated, frozen at −80 °C, and stored. After all samples were collected, HE4 levels were measured using an enzyme immunometric assay kit (Fujirebio Diagnostics, Inc., Malvern, Sweden) following the manufacturer's instructions.

Statistical analysis

SPSS v20 was used for the statistical analysis (SPSS Inc., Chicago, United States). The normality of the parameters was assessed using the Kolmogorov–Smirnov test. One-way ANOVA and post hoc tests were used to compare normally distributed data (i.e., the comparison of HE4 levels among pregnant women with preeclampsia, pregnant women without preeclampsia, and healthy non-pregnant women). Kruskal–Wallis tests were used to compare non-normally distributed data. Data were recorded as the mean (standard deviation [SD]), and $P < 0.05$ was considered to be significant.

Results

The clinical, biochemical, and obstetric characteristics of the groups are shown in Tables 1 and 2. There was no statistically significant difference between the three groups in terms of age, number of pregnancies, pregnancy end week, hemoglobin or hematocrit values, or international normalized ratios. However, the preeclampsia group had the highest systolic ($P = 0.001$) and diastolic ($P = 0.002$) blood pressures, urogram ($P < 0.001$) and 24-hour urine protein levels ($P < 0.001$), aspartate aminotransferase ($P < 0.001$) and alanine aminotransferase ($P < 0.001$) levels, creatine levels ($P < 0.001$), and lactate dehydrogenase levels ($P < 0.001$), and these levels were statistically significant. Platelet values were lowest in the preeclampsia group and these were also statistically significant ($P = 0.007$).

Table 1: Comparison of the demographic characteristics among the groups

Descriptive Characteristics mean (SD) (min-max)	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Age (years)	27.25 (6.27) (18-39)	33.65 (6.63) (24-44)	30.45 (6.00) (20-40)	0.010
Number of pregnancies (gravida)	2.65 (1.98) (1-8)	3.50 (1.93) (1-8)	3.45 (2.58) (1-10)	0.277
Pregnancy end week	36 (1.52) (34-39)	36.10 (2.07) (34-40)	-	0.890
Systolic blood pressure at admission (mm Hg)	165.50 (18.77) (140-190)	112.00 (12.81) (90-135)	109.50 (13.46) (90-135)	0.001
Diastolic blood pressure at admission (mm Hg)	99.00 (13.33) (70-120)	68.50 (9.33) (50-80)	64.5 (8.87) (50-80)	0.002

Min: Minimum, Max: Maximum, SD: standard deviation

The statistical analysis showed that HE4 levels were significantly lower ($P = 0.002$) in women with preeclampsia (38.76 pmol/L) than in pregnant women without preeclampsia (64.40 pmol/L) and healthy non-pregnant women (59.98 pmol/L) (one-way ANOVA). Although the healthy non-pregnant women's HE4 levels were lower than the healthy pregnant women's levels, the finding was not significant ($P = 0.477$). The comparison of HE4 levels among the three groups is shown in Table 3.

Table 2: Comparison of the laboratory findings among the groups

Descriptive Characteristic mean (SD) (min-max)	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Urogram protein (+1 - +4)	1.80 (1.15) (0.00-4.00)	0.30 (0.57) (0.00-2.00)	0.25 (0.55) (0.00-2.00)	<0.001
Urogram protein n (%)				
0	3 (15.0%)	15 (75.0%)	16 (80.0%)	<0.001
+1	5 (25.0%)	4 (20.0%)	3 (15.0%)	
+2	6 (30.0%)	1 (5.0%)	1 (5.0%)	
+3	5 (25.0%)	0 (0.0%)	0 (0.0%)	
+4	1 (5.0%)	0 (0.0%)	0 (0.0%)	
24-hours urine protein (g/L)	1.76 (1.34) (0.30-5.10)	0.23 (0.16) (0.10-0.60)	-	<0.001
Hemoglobin (g/dL)	12.21 (2.27) (7.8-16.6)	11.45 (2.05) (7.0-15.0)	11.8 (0.85) (10.0-13.7)	0.757
Hematocrit (%)	35.0 (5.50) (25.9-45.6)	33.57 (6.00) (21.7-43.4)	35.14 (2.10) (30.5-38.7)	0.630
Platelet (µL)	164,900 (92,109) (47,000-365,000)	209,000 (68,372) (110,000-365,000)	249,850 (59,823) (161,000-365,000)	0.007
Aspartate aminotransferase (IU/L)	269.55 (293.44) (72-1113)	30.30 (13.06) (10-58)	19.60 (6.09) (10-35)	<0.001
Alanine aminotransferase (IU/L)	159.80 (105.10) (51-481)	31.40 (10.65) (11-50)	15.80 (5.90) (10-31)	<0.001
Lactate dehydrogenase (IU/L)	1069.45 (676.16) (512-3051)	220.15 (44.60) (132-284)	235.15 (61.04) (158-384)	<0.001
International normalized ratio (INR)	0.99 (0.15) (0.76-1.37)	1.10 (0.32) (0.85-2.16)	0.99 (0.09) (0.87-1.19)	0.722
Creatine (mg/dL)	0.97 (0.90) (0.50-4.18)	0.74(0.27) (0.49-1.62)	0.47 (0.14) (0.29-0.85)	<0.001

Table 3: Comparison of human epididymis protein 4 levels among the groups

	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Mean HE4 level mean (SD) (min-max)	38.76 (12,01) (17.65-65.34)	64.40 (12.69) (29.39-89.15)	59.98 (11.15) (41.76-81.75)	0.002

HE4 protein level: pmol/L, n: number of patients

Discussion

The present study is the first to investigate this topic. We aimed to determine the role of the HE4 protein in the pathogenesis of preeclampsia. We found that the mean HE4 levels were lowest in pregnant women with preeclampsia compared to healthy pregnant women and non-pregnant women. Our literature search revealed a lack of comparative research. Only a few studies have determined HE4 levels during pregnancy, and although a recent study evaluated HE4 protein levels in pregnant women with HELLP syndrome [14], there are currently no studies that have evaluated HE4 levels in women with preeclampsia. Furthermore, it should be noted that because of the variable range in the HE4 protein levels between studies caused by differences in the kits and devices used for measurement, the results were not comparable among studies.

We found that HE4 levels were higher in the healthy pregnant group than in the non-pregnant group; however, this difference was not significant. In a study by Gucer et al. [15], no significant differences were reported between pregnant and non-pregnant patients. In another study including 1,101 healthy non-pregnant and 67 pregnant women, Moore et al. reported that serum HE4 levels were significantly lower in pregnant women than in premenopausal, non-pregnant women, a finding contrary to ours. They also found that serum HE4 levels were significantly lower in premenopausal women than in postmenopausal women [16]. Age had no effect on our study results, however, as there was no significant difference in the mean age among our groups.

In another study, Uslu et al. [11] found that HE4 levels decreased in the first and second trimesters, but there was no significant difference in the levels of HE4 protein in the third trimester compared to pre-pregnancy levels. Unlike Uslu et al., we found that HE4 protein levels were higher in the healthy pregnancy group than in the non-pregnant (pre-pregnancy) group. Wang et al. [17] reported that HE4 levels did not change significantly in the first and second trimesters compared with the levels in non-pregnant patients. They further indicated that HE4 levels were significantly higher in the third trimester compared with the levels in non-pregnant women. Our results were similar to these, but the difference we found when comparing the HE4 protein levels between the healthy pregnant group and the non-pregnant group was not significant.

Gasiorowska et al. [18] found that HE4 levels significantly changed with age, which is supported by the literature [16, 18, 19], and they found a significant correlation between smoking and serum HE4 levels [18]. We excluded smokers from the study, so we could not assess that variable. In the same study, Gasiorowska et al. [18] compared HE4 protein levels among the first, second, and third trimesters and reported that while there was no significant difference between the first and second trimesters, there was a significant increase in the third trimester.

The literature supports the idea that HE4 protein levels are affected by age and gestational length. However, neither of these affected our results because all pregnancies in our study were over 34 weeks, and age was not statistically different among all participants. Thus, our study was not affected by any factor that could affect HE4 protein levels, such as age and gestational week.

Kurdoglu et al. [20] researched laminin receptor 1, an extracellular matrix protein, to see if it played a role in the pathophysiology of preeclampsia. Many studies have shown that laminin receptor 1 is involved in cell adhesion, invasion, and migration and is effective on protease activity (like the HE4 protein); in addition, it was shown to have a role in malignancies and tumor metastases [20-22]. Kurdoglu et al.'s [20] results were statistically significant and showed that laminin receptor 1 was less expressed in preeclamptic placentas, but the severity of the disease was not related.

In a recently study conducted by Cam et al. [14] HE4 protein levels in pregnant women with HELLP syndrome, an extremely advanced form of preeclampsia were compared, with healthy pregnant women. Similar to our study, they found that the mean HE4 protein levels in HELLP syndrome patients were lower, but in contrast to our study, the result was not statistically significant.

Limitations

In this study, we did not investigate the relationship between the severity of preeclampsia and HE4 protein levels, nor did we measure placental HE4 protein levels. This is a limiting factor; thus, further extensive studies are required.

Conclusion

To adequately understand preeclampsia and predict its complications, the identification of high-risk women could help minimize undesirable fetomaternal consequences. Considering that the diagnostic criteria for the condition are frequently reviewed, we believe that a predictive biochemical marker would help improve pregnancy outcomes. Our study suggests that a decrease in HE4 protein levels can be used as a new biomarker to predict the development of preeclampsia. However, much more comprehensive research is needed in the future, as well as studies with a much higher number of patients.

References

1. McCarthy FP, Ryan RM, Chappell LC. Prospective biomarkers in preterm preeclampsia: A review. Pregnancy Hypertension. 2018;14:72-8.
2. Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. Eur J Obstet Gynecol Reprod Biol. 2013;170(1):1-7.
3. Fisher SJ. Why is placentation abnormal in preeclampsia? American Journal of Obstetrics and Gynecology. 2015;213(4 Suppl):S115-22.
4. Lim KH, Zhou Y, Janatpour M, McMaster M, Bass K, Chun SH, et al. Human cytotrophoblast differentiation/invasion is abnormal in pre-eclampsia. The American Journal of Pathology. 1997;151(6):1809-18.
5. Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, et al. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. The Journal of Cell Biology. 1991;113(2):437-49.
6. McKinnon B, Mueller MD, Nirgianakis K, Bersinger NA. Comparison of ovarian cancer markers in endometriosis favours HE4 over CA125. Molecular Medicine Reports. 2015;12(4):5179-84.
7. Yilmaz EP, Kumtepe Y. Endometrial and ovarian cancer with MR imaging importance of serum HE4 and CA 125 levels in the extent of disease at evaluation. The Eurasian Journal of Medicine. 2016;48(3):192-8.
8. Jia LT, Zhang YC, Li J, Tian Y, Li JF. The role of human epididymis protein 4 in the diagnosis of epithelial ovarian cancer. Clin Transl Oncol. 2016;18(3):233-9.
9. Galgano MT, Hampton GM, Frierson HF, Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. Modern Pathology : An Official Journal of The United States and Canadian Academy of Pathology, Inc. 2006;19(6):847-53.
10. Chen P, Yang Q, Li X, Qin Y. Potential association between elevated serum human epididymis protein 4 and renal fibrosis: A systemic review and meta-analysis. Medicine. 2017;96(36):e7824.
11. Uslu B, Dogan S, Özdem S, Şimşek T. Serum concentrations of HE4 and Ca125 in uncomplicated pregnancies: a longitudinal study. J Obstet Gynaecol. 2020;40(1):70-6.
12. Bellos I, Pergialiotis V, Loutradis D, Papapanagiotou A, Daskalakis G. Serum CA-125 levels in preeclampsia: A systematic review and meta-analysis. Int J Clin Pract. 2019;73(10):e13380.
13. Gestational hypertension and preeclampsia: ACOG practice bulletin summary, number 222. Obstet Gynecol 2020;135(6):1492-5.
14. Cam T, Cimilli Senocak GN, Ozturk N, Topdagi Yilmaz EP. May human epididymis 4 protein play a role in the etiopathogenesis of hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome? J Obstet Gynaecol Res. 2021 Jul;47(7):2324-8. doi: 10.1111/jog.14808.
15. Gucer F, Kiran G, Canaz E, Kilinc M, Ekerbicer HC, Avci F, et al. Serum human epididymis protein 4 can be a useful tumor marker in the differential diagnosis of adnexal masses during pregnancy: a pilot study. Eur J Gynaecol Oncol. 2015;36(4):406-9.
16. Moore RG, Miller MC, Eklund EE, Lu KH, Bast RC, Jr., Lambert-Messerlian G. Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age. Am J Obstet Gynecol. 2012;206(4):349.e1-7.
17. Wang Z, Zhou F, Xiao X, Ying C. Serum levels of human epididymis protein 4 are more stable than cancer antigen 125 in early and mid-term pregnancy. J Obstet Gynaecol Res. 2018;44(11):2053-8.
18. Gasiorowska E, Kluz T, Lipski D, Warchoł W, Tykarski A, Nowak-Markwitz E. Human epididymis protein 4 (HE4) reference limits in polish population of healthy women, pregnant women, and women with benign ovarian tumors. Dis Markers. 2019;2019:3890906.
19. Bolstad N, Øijordsbakken M, Nustad K, Bjerner J. Human epididymis protein 4 reference limits and natural variation in a Nordic reference population. Tumour Biol. 2012;33(1):141-8.
20. Kurdoglu M, Kurdoglu Z, Ozen S, Kucukaydin Z, Bulut G, Erten R, et al. Expression of laminin receptor 1 in human placentas from normal and preeclamptic pregnancies and its relationship with the severity of preeclampsia. J Perinat Med. 2011;39(4):411-6.
21. Mecham RP. Receptors for laminin on mammalian cells. Faseb J. 1991;5(11):2538-46.
22. Morais Freitas V, Nogueira da Gama de Souza L, Cyreno Oliveira E, Furuse C, Cavalcanti de Araújo V, Gastaldoni Jaeger R. Malignancy-related 67kDa laminin receptor in adenoid cystic carcinoma. Effect on migration and beta-catenin expression. Oral Oncol. 2007;43(10):987-98.

The National Library of Medicine (NLM) citation style guide has been used in this paper.