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# Prokinetisin 1 preeklampsi belirteci olarak kullanılabilir mi?

# Can prokineticin 1 be used as a biomarker in preeclampsia?

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## ÖZ

**Amaç:** Prokinetisinler, anjiogenezde, hematopoezde ve üremede önemli rolleri olan bir grup peptiddir. Prokinetisin 1 (PROK1) kadın üreme sistemi gelişimini etkileyen en majör altgruptur. PROK1 sinsityotrofoblastlardan salınmakta olup anormal plasentasyona ve preeklampsiye (PE) yol açan ilk trimesterdeki extravillöz trofoblastik hücre göçünü engeller. Hipoksi, PROK1'in ekspresyonunu uyarır ve böylelikle preeklamptik gebelerde serum PROK1 düzeyi artar. Bu çalışmanın amacı, PROK 1'in bir PE belirteci olarak değerini ortaya koymaktı.

**Gereçler ve Yöntem:** Çalışmaya total olarak 84 gebe dahil edildi ve onların 43'ü preeklampsi tanısı almıştı. Kırk bir sağlıklı gebe kontrol grubuna dahil edildi. PE grubu; hafif PE (n=27) ve şiddetli PE (n=16) olmak üzere iki alt gruba ayrıldı. Yaklaşık 10 ml venöz kan her bir katılımcıdan toplandı ve lityum heparin içinde ayrıştırılarak, analiz edilinceye dek – 80 C'de saklanan serum elde edildi. Serum PROK1 ölçümleri enzim bağımlı immünosorbend tahlil ile (ELİSA) yapıldı. Tüm testler iki uçlu olup, $p<0.05$ iken istatistiksel olarak anlamlı kabul edildi.

**Bulgular:** Çalışmamızda iki grubun PROK1 düzeylerinde anlamlı bir fark bulamadık (115,66±22,41 vs 115,57±26,27; p=0,91). Gruplar arasında anne yaşı (yıl) ve başvuru sırasındaki gebelik yaşı açısından anlamlı bir fark saptanmadı. Preeklamptik grup hafif PE (n=27) ve ağır PE (n=16) olmak üzere iki alt gruba ayrıldı (Table 3). Hafif PE grubunda PROK1 düzeyi, şiddetli PE ve kontrol gruplarına göre daha düşüktü, fakat bu sapma istatistiksel olarak anlamlı bulunmadı.

**Sonuç:** Çalışmamızda, plasental anjiogenezi ve trofoblast invazyonunu etkilediği gerçeğinden yola çıkarak PROK1'in PE etiyolojisinde rol oynadığı düşünüldü. Çalışmamızda PROK1'in, PE belirteci olarak işaret eden istatistiksel anlamlı bir sonuca ulaşılmasa da, bu belirtecin preeklampsideki rolünü belirlemek için daha büyük ölçekli ileri çalışmalara ihtiyaç vardır.

**Anahtar kelimeler:** preeklampsi, prokinetisin 1, belirteç

## ABSTRACT

**Aim:** Prokineticins are a group of peptides which play important roles in angiogenesis, hematopoiesis and reproduction. Prokineticin 1 (PROK1) is reported to be the major subgroup affecting the female reproductive development.

PROK1 is secreted from the syncytiotrophoblasts and it prevents extravillous trophoblastic cell migration in the first trimester which predispose to the abnormal placentation and preeclampsia (PE). Hypoxia provokes the expression of PROK1 therefore its serum levels increase in pregnants with PE. The aim of the present study was to reveal the value of PROK1 as a marker of PE.

**Materials and Method:** A total of 84 pregnant women were admitted to the study, and 43 of them were diagnosed with preeclampsia. 41 healthy pregnant women were included in the control group. The preeclampsia group was divided into two subgroups as mild PE (n=27) and severe PE (n=16).

About 10 ml of venous blood was collected from each participant and dispensed into lithium heparin, and serum was obtained, which were stored at – 80 C until analyzed. Serum PROK1 measurements were performed by using an enzyme-linked immunosorbent assay (ELİSA). All tests were two-tailed, and $p<0.05$ was considered to be statistically significant.

**Results:** In our study we did not find any significant difference in PROK1 levels of the two groups. (115.66±22.41 vs115.57±26.27; p=0.91). Groups showed no significant differences in maternal age (years) and gestational age at admission. The preeclamptic group was separated into the two groups as mild PE (n=27) and severe PE (n=16) (Table 3). The prokineticin 1 level was lower in the mild preeclamptic group than the severe and control groups and this declination did not reach statistical significance.

**Conclusion**: PROK1 is thought to play role in the etiology of PE regarding the fact that this peptide affects the trophoblast invasion and placental angiogenesis. Although our present study did not reach a result indicating PROK1 as a predictor of PE, larger scaled studies are needed to clarify the role of this factor in PE.

**Keywords**: preeclampsia, prokineticin 1, predictor

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## INTRODUCTION

Prokineticins are a group of peptides which were first revealed to be functioning in the gastrointestinal tract (1). They are classified into three groups: PROK1 (prokineticin1), PROK2 and PK2L (longer form of PROK2) (1,2). They play important roles especially in angiogenesis (2), hematopoiesis (3) and reproduction (4,3,5). They also shown to be related with immune response (3) neuronal activity (4). PROK1, a peptide of 86 amino acids, is reported to be the major prokineticin affecting the female reproductive development (6).

PROK1 is mainly found in hypothalamus, adrenal cortex, ovaries, testicles and placenta. It is also named as EG-VEGF (endocrine gland-derived vascular endothelial growth factor) because it plays important role in angiogenesis like the vascular endothelial growth factor (VEGF) (7).

PROK1 is secreted from the syncytiotrophoblasts and it is very vital for human placental growth and maturation. It affects the invasion of trophoblast and villous growth (5). Furthermore the proliferation of fetal endothelial cells is affected by PROK1 (8). PROK1 prevents extravillous trophoblastic cell migration in the first trimester and stimulates their proliferation (9). Defect in spiral artery remodelling and trophoblast invasion and the failure of cytotrophoblast cells in penetrating the myometrial segment of the spiral arteries are the underlying factors of the abnormal placentation and preeclampsia. Hypoxia provokes the expression of PROK1 therefore its serum levels increase in pregnants with Preeclampsia (PE) (9).

The development of PE may not be unexpected following a problem in the expression of PROK1. The present study was performed to clarify whether PROK1 could be considered as a predictive marker of PE or not.

## MATERIALS AND METHODS

This case- control study was conducted in the Department of Obstetrics and Gynecology,Ümraniye Research and Education Hospital, between October 2016 and September 2017.

A total of 84 pregnant women were admitted to the study,and 43 of them were diagnosedwith preeclampsia;whereas 41 healthy pregnant women occupy the control group. The preeclampsia group was divided into two subgroups as mild PE (n=27)and severe PE (n=16). The diagnosis was based on the definitions of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy [10]. It is defined as a blood pressure (BP) of >140mmHg systolic or >90mmHg diastolic in a women who was normotensive before 20 weeks of gestation and accompanied by proteinuria (0.3 g/24 h, which correlates with >30 mg/dl or >1 + reading on dipstick in a random urine determination with no evidence of urinary tract infection). Forty-one healthy pregnant women were normotensive and had no proteinuria.

All the participants were in the third trimester and not in active labor, and none had rupture of membranes. Exclusion criteria were determined multiple gestation, chromosomal or congenital fetal abnormality, chronic hypertension, pregnancy -induced hypertension, molar pregnancy, type 1 diabetes mellitus, maternal renal disease, PE history, and morbid obesity. In addition, alcohol consumption, smokers, and who had any medical/ surgical intervention or on any medication to manage PE before enrolment were also excluded. The study protocol was approved by the Ethical Committee of Ümraniye Research and Education Hospital (no: 21192). All participants gave written informed consent.

Demographic characteristics and obstetrics outcomes were recorded. Age, gravida, parity, education level on admission were recorded in the database. Gestational age was calculated by the first day of their last menstrual period and confirmed by first-trimester ultrasound scanning of the crown-rump length (CRL). After at least 10 minutes of rest, BP was obtained in the left-side lying position using a mercury sphygmomanometer. Korotkoff phases 1 and 5 were used as recommended (11). About 10 ml of venous blood was collected from each participant and dispensed into lithium heparin, and serum was obtained. Then centrifugation was done at 2000-3000 RPM for 20 minutes within 30 minutes of collection . Samples were stored at – 80 C until analyzed. Samples were brought to room temperature before starting the assay. Serum EG-VEGF measurements were performed by using an enzyme-linked immunosorbent assay (ELİSA). (Hangzhou Eastbiopharm, Hangzhou). We added sample and ELİSA reagent into each well and incubated for 1 hour at 37 C . The plates were washed 5 times. We added substrate solution A and B and incubated for 10 minutes. After that we added stop solution and color developed. We red the Optical density value within 10 minutes. In addition, 5 ml of random urine (midstream ) was collected for urinalysis using the dipstick method. After proteinuria was detected in the dipstick method, we confirmed the results with a 24-hour protein assessment.

Statistical analysis

Data analysis was done using SPSS, version 17.0. Comparison

of variables between groups was made by using a one-way analysis of variance (ANOVA) followed by a post-hoc test. The relationship between qualitative variables was determined by using Fisher's exact test. All tests were two-tailed, and $p<0.05$ was considered to be statistically significant. One-way analysis of variance (ANOVA) was to compare more than two normally distributed variables, while the Kruskal–Wallis test with the Bonferroni post hoc test was applied if the variables were not normally distributed. The independent-samples t -test was used to compare two groups in terms of normally distributed quantitative data. The Mann–Whitney U test was used if the distribution of the variables was not consistent with normality. Analysis of covariance (ANCOVA) was used to analyze the difference between groups, with gestational age at sampling date and maternal weight as covariates.

## RESULTS

Fourty three pregnant womenpresenting with PE were matched with 41 healthy women according to age and parity. Demographic characteristics and pregnancy outcomes of the patients were summarized in Table 1.Groups showed no significant differences in maternal age (years) and gestational age at admission (Table 1).

**Table 1:** Clinical characteristics of study participants

| | Control (n = 41) | Preeclampsia (n = 43) | p value |
|---|---|---|---|
| Age (year) | 28.63±5.4 | 30.12±6.5 | 0.26 |
| Gravidity (range) | 2 (1-7) | 2 (1-7) | 0.71 |
| Parity (range) | 1 (0-6) | 1(0-5) | 0.35 |
| Gestational week at delivery | 38.8 ± 1.43 | 35.93 ± 3.5 | <0.001 |
| BMI | 24.68 ± 5.4 | 25.65±3.2 | 0.32 |
| Fetal weight (gr) | 3262.8 ± 403.9 | 2806.8 ± 886.06 | 0.003 |
| Proteinuria | - | + | 0.01 |

Gestational week at delivery was significantly shorter in the preeclampsia group than the control group (38.8 ± 1.43 vs. 35.93 ± 3.5; $p<0.001$). Fetal weight in the preeclampsia group was significantly weightless than the control group (3262.8±403.9 vs. 2806.8± 886.06; $p=0.003$).

Prokineticin-1 levels were similar between study groups (115.66±22.41 vs115.57±26.27; $p=0.91$) (Table 2).

**Table 2:** Prokineticin-1, Platelet levels of all groups.

| | Control (n = 41) | Preeclampsia (n = 43) | p value |
|---|---|---|---|
| Prokineticin-1 (pg/ml) (mean ±SD) | 115.66±22.41 | 115.57±26.27 | 0.91 |
| Platelet ($10^9$ /L ) | 223.853.6±54.86 | 210.139.53±57.28 | 0.26 |

Platelet counts were similar in the two groups ($p=0.26$).

The preeclamptic group was separated into the two groups as mild PE (n=27) and severe PE (n=16) (Table 3).

**Table 3**: Prokineticin-1 levels between control, mild severe pre-eclampsia group

| | Control (n = 41) | Mild Preeclampsia (n=27) | Severe Preeclampsia (n=16) | P value |
|---|---|---|---|---|
| Prokineticin-1 (pg/ml) (mean ±SD) | 115.66±22.41 | 109.66±14.31 | 125.54±37.52 | 0.117 |

The prokineticin-1 level was lower in the mild preeclamptic group than the severe and control groups andthis declination did not reach statistical significance. This result can be related with the small number of participants of the two groups.

## DISCUSSION

PE is a life threatening disease that affects almost 10% of all pregnancies and characterized by high blood pressure and proteinuria developing in the second part of the pregnancy (10). It is known that PROK1 is a pro-angiogenic placental factor which is upregulated by hypoxia and hCG (11). PROK1 is thought to play role in the etiology of PE regarding the fact that this peptide affects the trophoblast invasion and placental angiogenesis. It is reported that serum PROK1 levels of non-pregnant women are approximately 50 pg/mL and are one fifth of the pregnant women in first trimester. It decreases to the level of 70 pg/mL at the second and third trimesters (9,12).

Hoffmann et al. revealed that the placenta was a great source of serum PROK1 and its levels were higher in patients with pre-eclampsia (9). Sergent et al. showed that preserving the high levels of PROK1 caused the occurence of pregnancy induced hypertension (13). They also suggested that these high levels of PROK1 in mice were related to the impairment of placental function and trophoblastic invasion. However in our study we did not find any significant difference in PROK1 levels of the two groups.

Petrik et al declared that in polycystic ovarian syndrome (PCOS), EG-VEGF mRNA expression occured in the theca interna and stroma of the ovary (14). Furthermore Ajonuma et al. suggested that PROK1 was a good predictor of ovarian hyperstimulation syndrome (OHSS) (15). Depending on these studies that showing the effect of PROK1 on angiogenesis in ovaries we tried to show that PROK1 might also affect the angiogenesis in placenta.

PROK1 was also shown to play an important role in the embryo implantation (16). Besides, it was reported that some mutations in PROK1 predisposed to ectopic pregnancies and even repeated miscarriages (17). Therefore this peptide was worth to investigate for another pregnancy complication, preeclampsia.

Goi et al. researched the relation between EG-VEGF and colorectal cancer and pointed the strong expression of EG-VEGF in these tumors (18). PROK1 (EG-VEGF) expression was also shown to be a good predictor of prognosis of the epithelial ovarian cancer (19). Hypoxia of the tumoral cells was shown to cause to the increase in PROK1 release, which was reported to be increased in ovarian cancer. Because hypoxia is also seen in PE, we investigated the relation of PROK1 and PE.

Recently, 7 et al. found elevated first-trimester serum PROK1 levels in patients whom developed PE and intrauterine growth retardation (IUGR) in the second and third trimesters (20). Sergent et al. reported that the circulating levels of PROK1 were higher in pregnants with IUGR (13). These studies in the literature reflect that PROK1 level increases in IUGR which is oftenly a consequence of PE.

The subject number may be seen as a limitation of our study. However the size of the study groups were calculated as sufficient for the power of the study. Anyway much more studies including greater number of participants are needed to clarify whether the increase in PROK1 levels is a cause or consequence of the disease. Another limitation was that, the study was a case control study. However to the best of our knowledge, our study is one of a few study in the literature evaluating the relationship between PROK1 and PE. Another strength of the present study is the fact that it was conducted in a single centre by a professional team.

Conclusion

Despite the fact that our present study did not reach a result indicating PROK1 as a predictor of PE, larger scale studies are

needed to reveal. Moreover further studies may be designed to identify the role of this factor in PCOS.

Disclosure:There is no conflict of interest between the authors.

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