ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 28(3): 499-503, 2023
DOI: 10.5505/ejm.2023.13911

Investigation of Kın of Irre-Like Protein Levels In Patients of Preeclampsia: A Case -Control Study

Yusuf Başkıran[1*], İbrahim Polat[2]

[1]*Van Eğitim ve Araştırma Hastanesi*
[2]*İstanbul Başakşehir Çam ve Sakura Şehir Hastanesi*

ABSTRACT

Preeclampsia is a hypertensive disorder of pregnancy that usually starts after the 20th week of pregnancy and affects 2-8% of pregnant women worldwide. In preeclampsia , glomerular endothelial damage, formation of podocituria and tubular hyperfiltration mechanisms play a role in the pathogenesis of proteinuria.
KIRREL is a protein from the nephrin adhesion family. Prevents podocyte damage. In this way, it maintains glomerular filtration and glomerular function.
The aim of the study is to show whether the levels of KIRREL an adhesion molecule protein, decrease in pregnant women with preeclampsia and to use it in the prediction of preeclampsia patients, to intervene in these patients beforehand.
A total of 80 pregnant women, 40 of whom were preeclampsia and 40 of whom were normotensive, who applied to the obstetrics and gynecology clinic of a tertiary hospital were included in our study. Our patients were diagnosed with preeclampsia according to the ACOG diagnostic criteria.
While the mean KIRREL level in the maternal blood of the pregnant women included in the study was 3.38 (mg/dl)in the study group, it was 6.46 (mg/dl)in the control group, and there was a significant difference between the groups. In the study group, the KIRREL level in maternal blood was lower than in normal pregnant women ($p = 0.377$).
In our study, serum KIRREL levels were found to be significantly lower in the study group compared to the control group. However, KIRREL levels have been evaluated as an inadequate test in the prediction of preeclampsia. This may be related to the fact that there are many factors affecting the development of preeclampsia.

Keywords : KIRREL protein, Preeclampsia, Proteinuria, Pregnancy

Introduction

Preeclampsia is a hypertensive pregnancy ilness that generally starts later the 20th week of pregnancy, influences 2-8% of pregnant women in the world (1,2). Preeclampsia; is a multisystemic disorder characterized by pregnancy-specific endothelial dysfunction and organ hypoperfusion secondary to vasospasm (3). Preeclampsia is a high systolic or diastolic blood pressure in two measurements taken at least 4 hours apart at rest in pregnancies who were normatensive before pregnancy and over 20 weeks of age. (SBP) ≥140 mmHg or (DBP) ≥90 mmHg)(Table 1) (4).

In a healthy pregnancy, kidney blood flow and glomerular filtration rate increase. The situation is reversed in preeclamptic pregnant women. The reason for this is estimated to be increased resistance in the renal afferent arteriole and deterioration in the glomerular endothelial structure. When the glomeruli of preeclamptic pregnant women were pathologically examined, it was known for many years that the endothelial layer was thickened and the windows on it were narrowed due to edema. In severe preeclamptic pregnant women, oliguria, that is, the total amount of urine made for 24 hours, is less than 500 ml. Renal pathologies observed in preeclamptic pregnant women are generally reversible. However, damage can reach permanent dimensions in pathologies such as renal failure and acute tubular necrosis resulting from hypovolemia due to excessive bleeding (5).

Proteinuria: It is the situation where the amount of protein in the 24-hour urine is 0.3 g or more, or the protein creatinine ratio in the spontaneous urine is 0.3 or greater. Generally, the daily proteinuria amount in preeclamptic pregnant women is below 5 g. In severe preeclampsia, there may be protein losses of 10 grams or more per day. The most common cause of severe proteinuria in pregnant women is the pathogenesis of preeclampsia. Glomerular endothelial damage, podocituria formation, and tubular hyperfiltration mechanisms play a role in the formation of proteinuria (6).

***Corresponding Author:** Yusuf Başkıran, Specialist Doctor, Van Training and Research Hospital, Van, Turkey,
Email: yusufbaskiran1@gmail.com, Phone: +90 (541) 952 34 68
ORCID ID: Yusuf Başkıran: 0000-0003-1123-6062, **İbrahim Polat:** 0000-0002-1418-634X
Received: 04.07.2022, **Accepted:** 29.01.2023

Table 1. ACOG Preeclampsia Diagnostic Criteria

Blood pressure	• In a pregnant woman with normal blood pressure, systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg in two measurements taken at least four hours apart after the 20th gestational week; • If systolic blood pressure is ≥ 160 mmHg or diastolic blood pressure is ≥ 110 mmHg, a diagnosis of hypertension can be confirmed within a short time (minutes) of timely initiation of antihypertensive therapy.
and	
Proteinuria	• ≥ 300 mg proteinuria in 24-hour urine OR • Protein/Creatinine Ratio ≥ 0.3 (each in mg/dL) • 1+ proteinuria in the measurement made with a Urine Stick (in cases where other quantitative measurements cannot be made)
Or	
In the absence of proteinuria, with the onset of either of the following with New-Onset Hypertension.	
Thrombocytopenia	• Platelet count < 100,000/microliter
Kidney failure	•Serum creatinine concentration >1.1 mg/dL or a doubling of serum creatinine concentration in the absence of another kidney disease.
Impaired Liver Enzymes	• Increased liver transaminases up to twice the normal concentration.
Pulmonary Edema	
Cerebral and Visual Symptoms	

Table 2. The Demographic Data of The Participants

	Study Group	Control Group	P value
Age (year)	30,05±6,28	27,09±_6,31	0,377
Gravida	2,72±1,61	2,57±1,25	0,967
Parity	1,19±1,41	1,23±0,85	0,662
Gestational age (week)	29,54±3,28	29,22±5,01	0,546
Systolic Blood Pressure (mm/Hg)	151,23±14,19	110,49±11,82	<0,001*
Diastolic Blood Pressure (Mm/Hg)	94,19±8,48	68,05±8,13	<0,001*

* Student-T test, Values in bold represent statistically significant

Preeclampsia is a pregnancy-specific condition with organ hypoperfusion secondary to endothelial dysfunction and vasospasm. Preeclampsia since 2013; In a normotensive woman, hypertension (HT) occurring after the 20th week has been stated to be accompanied by "proteinuria or end-organ damage". Very high blood pressure and signs and symptoms of end-organ damage constitute the severe character of the disease (7).

Proteinuria is an indicator of glomerular pathology. Detection of 300 mg or more protein in 24-hour urine and more than +1 proteinuria in at least 2 urine samples taken with an interval of 6 hours or more are required for the diagnosis of pathological proteinuria (8). Studies have found a weak correlation between the protein level detected by dipstick and the amount of protein in 24-hour urine. Therefore, the quantitiy of protein in the 24-hour urine should be the main determinant for proteinuria (9). Additionally, a protein/creatinine ratio of 0.3 mg or more in spontaneous urine indicates proteinuria. Since preeclampsia is a condition characterized by spasms in the renal vessels from time to time, varying amounts of proteinuria can be detected in different urine samples (10)

KIRREL (Kin of irre-like protein) is a human protein produced from the same gene as its name, also called NEPH1. KIRREL belongs to the NEPH protein family, which also includes other KIRREL proteins. The cytoplasmic portions of

Table 3: Comparison of KIRREL Levels Between Groups

	Study Group	Control Group	P value
KIRREL Level (mg/dl)	3,38 ±7,26	6,46 ±11,08	<0,03*

*Student-T test, Values in bold represent statistically significant

these proteins interact with a portion of podocin. The KIRREL protein extracellularly contributes mainly to the structure of the cleft diaphragm, while the cytoplasmic domain of the KIRREL protein has been shown to induce a signal that reorganizes the actin cytoskeleton (3,7,8). Recent studies have shown that KIRREL affects cells in kidney podocytes that provide size- and charge-selective ultrafiltration of blood, which can interact with nephrin (NPHS1) and tight junction protein 1. We examined the effect of impaired filtration on KIRREL protein in preeclampsia in this study.

Materials and Methods

Our study, which included normotensive and preeclamptic pregnant women, is a case-control study. We examined a total of 80 pregnant women, 40 of whom were healthy normotensive and 40 with preeclampsia, who applied to the tertiary gynecology and obstetrics clinic between July and October 2020. After the approval of the ethics committee with the decision numbered KAEK/2020.04.7, the research was started. The patients were informed in detail. Informed consent was read and signed.

The European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC) arterial hypertension management guideline published in 2013 was used as the source to accurately assess blood pressure measurement. The blood pressure of each case was evaluated according to the standard measurement technique. Optimal conditions for blood pressure measurement were provided. The blood pressure of each subject was measured after resting for at least 5 minutes in a sitting position with the arm at heart level. Measurements were evaluated from the brachial artery. The people who made the measurement were trained health personnel, and blood pressure measuring devices with appropriately sized cuffs were used. The guide prepared by the American College of Obstetricians and Gynecologists (ACOG) in 2013 was used to diagnose preeclampsia.

Blood samples belonging to pregnant women were taken from preeclamptic patients who applied to our hospital between the 20th and 41st weeks of pregnancy, at the time of diagnosis. Healthy pregnant women were selected from those who applied to the hospital from those who were in similar weeks to the patient population. Care was taken not to initiate any treatment when blood was taken from the healthy control group of pregnant women. After sterilization of the forearm antecubital region of each patient in a sitting position by wiping with alcohol cotton, 3 cc blood samples were collected into tubes without anticoagulant. These collected samples were centrifuged at 3000 rpm for 20 minutes. The maternal serum sample obtained as a result of the centrifugation process was transferred to the Eppendorf tube and recorded on behalf of the patient. Collected serum samples were kept in -80-degree cabinets in the medical genetics laboratory until analysis.

Serum KIRREL (NEPH1) concentrations were examined using a commercially available enzyme-linked immunosorbent assay (ELISA) commercial kit. To generate a standard optical density (OD) curve against KIRREL concentration, we inserted specimens, standard specimens, and biotin-labeled antibodies to micropores/cuvettes pre-coated with KIRREL antibody, and then OD values of standard specimens and specimens; It was detected with a microplate spectrophotometer (Smart Microplate Reader; USCN KIT INC.) tuned to a wavelength of 450 nm. The KIRREL concentration in the specimens was then determined by comparing the OD value of the specimens with the standard curve. KIRREL concentrations were measured in mg/dl.

Statistical Data: SPSS program was used in our study. The values between the study group and the control group were compared with the "Student-T test". Values between both groups were expressed as "mean ± standard deviation". A P value less than <0.05 was considered significant. ROC curve was also drawn for reliable determination of KIRREL levels in the prediction of preeclampsia.

Results

While 40 (50%) of 80 pregnant women in our study were preeclamptic, the patients in the

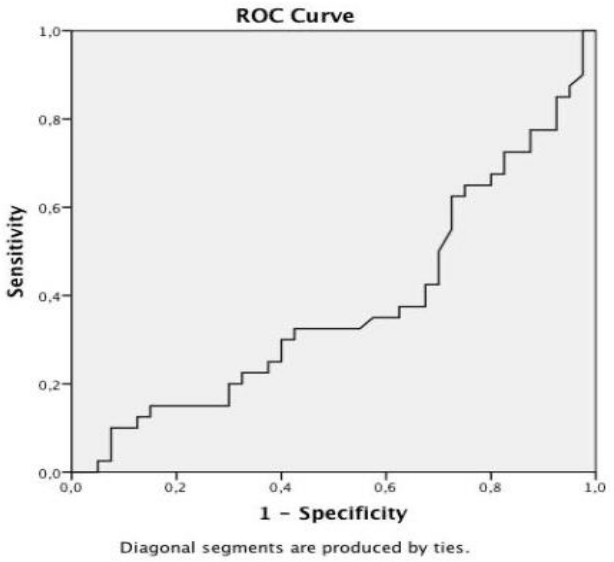

Fig. 1. ROC analysis of KIRREL levels in the prediction of preeclampsia.(p=0.478)

control group were normotensive (50%). Demographic data of our patients are shown in Table 2.

There was no significant difference between the groups in some values of the pregnant women included in the study, such as mean age, mean gravida, mean parity and mean gestational days. The mean systolic blood pressure and mean diastolic blood pressure of the study group were higher than the control group as we expected.

The mean KIRREL level in the maternal blood of the study group in the pregnant women included in the study was 3.38±7.26 (mg/dl), 6.46±11.08 (mg/dl) in the control group, and a significant difference was observed among the pregnant women (p.<0.03) (Table 3).

In the ROC curve drawn to evaluate the power of KIRREL levels in the prediction of preeclampsia, the area under the curve (AUC) value was calculated as 0.379 and the p value as 0.478 (Figure 1).

Discussion

Although the parameters used in the prediction of preeclampsia are insufficient today, blood pressure monitoring and blood and urine tests used in pregnancy follow-up are important markers in the prediction of preeclampsia and reduce preeclampsia mortality. In our study, which we started by considering the protein loss in the pathophysiology of preeclampsia, we measured the blood KIRREL level in the study and control group patients.

Since KIRREL is a very new protein, little research has been done on this subject before. We investigated the concentration of IRRE-like protein1 (KIRREL) in some tissues of the body, such as normal stomach tissue and gastric cancer tissue, and tried to find a semantic difference. The results proved that the expression level of KIRREL mRNA was higher in gastric cancer tissues than in other tissues.

KIRREL mRNA levels have been shown to be significantly increased by gastric cancer stage. Thus, overexpression of KIRREL has shown that these patients have a poor prognosis (11). KIRREL levels were also found to be lower in the study group compared to the control group in our study.

In another study, the interaction of ISD (isodesmosine) on NEPH1 (KIRREL) and ZO-1, which are effective in kidney filtration, were shown. The interactions of NEPH1 and ZO-1 are a crucial part of the kidney filtration system. Since injury may cause this interaction to be lost, it has been hypothesized that maintaining this interaction might preserve kidney function. Podocytes treated with ISD have been shown to be resistant to injury-induced loss of transepithelial permeability, i.e. impaired renal function. Likewise, this time in a study on mice and zebrafish, it was shown that ISD protects kidney function due to injury (12). Since the level of NEPH1 (KIRREL) was significantly low in our study group and it was thought to be associated with renal dysfunction, it is thought that it can be used as an auxiliary parameter in the selection of drugs to be used during pregnancy.

In another study, the anatomical and functional similarity of fly nephrocytes to human nephrocytes was shown. Notably, both cell types have been shown to have a known filtration diaphragm and in the absence of the KIRREL protein, the nephrocyte diaphragm is lost in flies (13). Similarly, in our study, the low values in the study group show the connection between KIRREL and kidney functions.

In another study, concentrations of nephrin proteins were measured in certain kidney diseases. In some glomerulonephritis, Adriamycin-induced nephropathy (ADR) and puromycin aminonucleoside nephrosis (PAN), total Nefl amount in podocytes was found to be decreased. The amount of nephrin was decreased in some nephropathies, but the amount of nephrin was not changed in focal segmental glomerulosclerosis (FSGS). Accordingly, since the level of nephrin (but not Neph) did not change in FSGS, it was

thought that Nephl might play a role in the disruption and pathogenesis of the complex (3). In our study, we saw that the KIRREL level decreased in the study group.

Despite the statistically significant decrease in KIRREL level in patients with preeclampsia, KIRREL levels are considered to be an inadequate test in the prediction of preeclampsia because the AUC value is below 0.6, and more research is needed.

In this study, we measured serum KIRREL concentrations in the study group and the other group and found that they were lower. However, we considered KIRREL concentrations to be an inadequate test for predicting preeclampsia. This may be related to the presence of many factors affecting the development of preeclampsia. There are very few studies on KIRREL levels in the literature and more studies are needed.

As a result, we think that the KIRREL serum level, which was found to be significant in our study, can be used as an auxiliary parameter to support the preliminary diagnosis, especially in preeclamptic pregnant women who have difficulty in diagnosis, and we think that it is a parameter that can be a precursor to further research on this subject. However, due to the statistically insufficient level of KIRREL protein, which we found significant in preeclamptic pregnant women, it was evaluated as an insufficient test in the prediction of preeclampsia, and more comprehensive studies are needed on this subject.

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