Full study protocol and statistical analysis plan

Official Title of the study:

An analysis soluble endoglin and matrix metalloproteinase 14 with Elisa method in the diagnosis and severity of early/late-onset preeclampsia

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1. Participant Flow

Recruitment Details

The study was conducted at Cengiz Gokcek Women's and Children's Hospital, Gaziantep, Turkey at department of obstetric and gynecology between date of January 2018 and December 2018. The study protocol was designed according to the Declaration of Helsinki, and the institutional ethical review board of Gaziantep University approved the study (Reference number: 2018/91). The study was conducted with 30 late-onset preeclampsia patients as group 1 (gestational age $\geq$ 34 weeks), 33 patients with normal pregnancies as group 2 (gestational age $\geq$ 34 weeks), 31 early-onset preeclampsia patients as group 3 (gestational age < 34 weeks), and 31 patients with normal pregnancies as group 4 (gestational age < 34 weeks). Finally, The authors consecutively recruited 61 pregnancies complicated with preeclampsia, and 64 healthy pregnancies will select for the control group. All participants included in the study gave oral and written informed consent.

Pre-assignment Details

The authors firstly were assessed the recruited people to ensure meeting the inclusion and

exclusion criteria.

The inclusion criteria were maken according to the official documents.

Arm/Group Information *

There are four groups in the study.

Arm/Group Title *

Preeclampsia groups = Group 1, group 3
Control groups= Group 2, group 4

2. Baseline Characteristics

<mark>In the analysis of variables, t-test was used for comparison of two groups and one-way analysis of variance was used for comparison of three groups. In addition, the demographic information of the variables were determined by frequency and percentage analyzes. The Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 22.0, IBM Corp., NY, USA).</mark>

3. Outcome Measures

Every woman in the study population underwent obstetric ultrasound examination and fetal-maternal assessment was carried out by one of the authors. Obstetric anamneses were obtained from all subjects. The demographic data for gestational age, gravidity, parity, maternal age and BMI was recorded. Maternal venous blood samples were taken for measurement of serum MMP-14 and s-ENG levels after the diagnosis of early/late onset preeclampsia in outpatient clinic. These samples quickly centrifuged at 1,500 g for 10 min, plasma and serum samples were separated and serum samples were stored at -80 C until the day of measurement. The participants with early/late onset preeclampsia were hospitalized. In early onset preeclampsia, a betamethasone injection was administered immediately after hospitalization. The pregnancy was immediately terminated in emergencies arising from maternal or fetal causes. Otherwise, blood pressure was measured every four hours during periods of rest. Hypertension can be confirmed within a shorter interval in patients with blood pressure ≥110mm Hg diastolic and ≥160mm Hg systolic in order to give timely antihypertensive treatment. Delivery should be postponed for at least 24-48 hours if maternal/fetal status permit. A betamethasone injection for lung maturation (two dosages of 12 mg at 24 h intervals) was given within the said period. The control group's samples were obtained during routine obstetrical care examination in the third trimester of pregnancy. Then these pregnant women were followed-up until delivery. The participants with late onset preeclampsia (LOPE) were also hospitalized and terminated. The control groups' samples obtained during the routine obstetrical care examination in the third trimester of pregnancy. Then these pregnant women were followed-up until the delivery. Four groups compared in terms of maternal age, BMI, gravida, parity, week of gestation, systolic/diastolic blood pressure, total protein in spot urine sample, white blood cell (WBC) count, hemoglobin, platelet count, blood urea nitrogen, creatinine, liver function tests (AST, ALT), MMP-14, s-ENG, and infant weight at delivery. The s-ENG level was measured with a commercially avaliable ELISA kits which is produced to detect human s-ENG level with a high sensitivity and specificity (Rel Assay Diagnostics, Gaziantep, Turkey). The measurements were done in accordance with company's protocol. The kit uses Sandwich-ELISA principle. Serum MMP-14 levels were determined using a commercially available ELISA kit, the Human MMP14 ELISA Kit (Rel Assay Diagnostics, Gaziantep, Turkey),

according to the manufacturer's instructions. Human MMP-14 ELISA kit is based on the principle of biotin double antibody sandwich technology.

4. *Endpoints of the study:*

The primary endpoint in this analysis is to evaluate the value of serum matrix metalloproteinase-14 levels (ng/ml) in preeclampsia patients and also address its relationship with its severity.

The secondary endpoint in this analysis is to evaluate the value of serum soluble endoglin levels (ng/ml) in preeclampsia patients and also address its relationship with its severity.

5.Limitations and strengths:

The main limitation of our study is the small number of patients. We do not have pre-pregnancy BMI of patients and controls. Levels of s-ENG and MMP-14 could also be measured in different compartments such as placenta, umbilical cord serum and amniotic fluid.
As for the strengths of the study, none of the patients had any treatments for preeclampsia and active labor

6. Certain Agreements

The authors declare that they have no conflict of interest.

7. Results Point of Contact

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