Full study protocol and statistical analysis plan

Official Title of the study:

Analyses of Interleukin-6, Presepsin and Pentraxin-3 in the diagnosis and severity of late-onset preeclampsia

Date of the document:

October 2019.

Contents:

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 June 2018 and January 2019. 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: 2018/393). The investigators included subjects consisted of women with a singleton pregnancy who were diagnosed as having late-onset preeclampsia between 34+0 and 41+0 weeks of gestation. Pregnant women with uncomplicated pregnancies were randomly selected to serve as controls. The study was conducted with 44 late-onset preeclampsia patients as study group and 44 patients with normal pregnancies as 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 two groups in the study.

Arm/Group Title *

Preeclampsia group
Control group

2. Baseline Characteristics

The normal distribution of data is analysed with Kolmogorov Smirnov ve Shapiro Wilk tests. Student t test was used for variables that normally distributed for gorup comparison(L-PrE/kontrol) and Mann Whitney U test was used for variables that is not normally distributed. Also, Sperasman correlation was used for relationship of non-normally distributed variables with each other. The ROC analysis was applied for determination of cut-off points for IL6, presepsin ve pentraxin-3 variables. SPSS for Windows 22.0 ve Medcalc programs were used for statistical analysis. $P<0.05$ was accepted as statistical significance.

3. Outcome Measures

Every pariticipant in the study population underwent obstetric ultrasound examination and fetal-maternal assesment was carried out by one of the investigators. The obstetric anamnesis were obtained from all pariticipants. The demographic data like age, gravidity, parity, body mass index (BMI) and gestational age were recorded. The gestational age was determined by calculation from the last menstrual period and supported by the ultrasonography measurements at first trimester of gestation. Maternal venous blood samples were taken for measurement of IL-6, presepsin and pentraxin-3 levels after the diagnosis of L-PrE in outpatient clinic. These samples quickly centrifuged at 1,500 g for 10 min, plasma samples were seperated and stored at -80°C until the day of measurement. All patients with L-PrE were also hospitalized and their pregnancies were terminated. The samples of the control groups were obtained during the routine obstetrical care examination in the third trimester of pregnancy. Then these pregnant women were followed-up until the delivery. Both groups were compared in terms of maternal age, BMI, week of gestation, gravida, parity, live born, systolic/diastolic blood pressure, total protein in spot urine sample, hemoglobin, hematocrit, platelet count, white blood cell count, neutrophil, lymphocyte, neutrophil-to-lymphocyte ratio (NLR), urea, uric acid, albumin, blood urea nitrogen, creatinine, liver function tests (AST, ALT), lactic acid dehydrogenase, IL-6, presepsin, pentraxin-3, and infant weight at delivery. Neutrophil-to-lymphocyte ratio (NLR), which is the ratio of the neutrophils and lymphocytes in blood sample at admission, which can accept a marker of systemic inflammation. Small for gestational age (SGA) neonate was defined as birth weight <10th percentile for gestational age with Turkey's national nomogram as the reference for fetal growth. The maternal plasma presepsin levels were determined using a commercially available ELISA kit, the human presepsin ELISA Kit (Rel Assay Diagnostics, Gaziantep, Turkey), according to the manufacturer's instructions. Human presepsin ELISA kit is based on the principle of biotin double antibody sandwich technology. IL-6 levels in blood samples were measured by using the ELISA method (DIAsource ImmunoAssays SA, Nivelles, Belgium) in accordance with the method described by the manufacturer. Pentraxin-3 levels were measured by using the ELISA kits in accordance with the manufacturer's instructions (Sunredbio, Shanghai, PRC).

4. ***Endpoints of the study:***

The primary endpoint in these analyses was IL-6, presepsin and pentraxin-3 levels in L-PrE group and control group.

The secondary endpoint was the IL-6, presepsin and pentraxin-3 levels in mild L-PrE group and severity L-PrE group.

Tertiary endpoint was the IL-6, presepsin and PTX-3 levels in SGA group and non-SGA group.

5.Limitations and strengths:

This study have some limitations. It was a small, observational and single-centered study. These three inflammatory markers were measured only during admission, we could not assess the changes in IL-6, presepsin, and pentraxin-3 in response to treatment due to lack of serial measurements.

We demonstrated, significant relations of all three elevated new inflammatory markers with both the presence and the severity of PrE at the same time in this study. Another strength of our study was that the women with PE had no pharmacological treatment. Because the antihypertensive drugs lead to the decrease in presepsin level due to the antiinflammatory effect.

6. Certain Agreements

The authors declare that they have no conflict of interest.

7. Results Point of Contact

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