Full study protocol and statistical analysis plan

Official Title of the study:

Serum Thiol/Disulphide Homeostasis level and its correlation with the severity of preeclampsia

Date of the document:

April 2019.

Contents:

1. Participant Flow

Recruitment Details

This was an observational prospective cohort study conducted at Obstetrics and Gynecology Department of Cengiz Gokcek Obstetrics and Children's Hospital between April and October 2019. One hundred women will enrolled in the study in two groups. We will consecutively recruit 50 pregnancies complicated with preeclampsia, and 50 healthy pregnancies will select for the control group. All patients will gave their oral and written informed consent before their inclusion in the study.

Pre-assignment Details

We firstly will assess the recruited people to ensure meeting the inclusion and exclusion criteria.

The inclusion criteria are made according to the official documents.

Arm/Group Information *

There are two groups in the study.

Arm/Group Title *

Preeclampsia group = Group A
Control group= Group B

2. Baseline Characteristics

Frequency and percentage analysis will be used for some variables during data analysis. In addition, independent samples t-test (Student's t-test) will be used for comparison of the thiol/disulfide levels by the variable groups formed. The SPSS (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. NY, USA) statistical program will be used for all statistical calculations.

3. Outcome Measures

Every women in the study population will undergo obstetric ultrasound examination and fetal-maternal assessment will carry out by one of the authors. The obstetric anamnesis will obtain from all subjects. The dempograhic data like age, gravidity, parity, BMI and gestational age will record. Maternal venous blood samples will take for measurement of thiol and disulphide levels after the diagnosis of preeclampsia in outpatient clinic. These samples quickly will centrifuge at 1,500 g for 10 min, serum samples will separate and store at -80 C until the day of measurement. All patients with early onset preeclampsia will hospitalize. A betamethasone injection will administer immediately after hospitalization. The pregnancy will immediately terminate in emergencies arising from maternal or fetal causes. Otherwise, blood pressure will measured every four hours during periods of rest. Hypertension can will 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 will postpone 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) will

give within the said period. The control group's samples will obtaine during routine obstetrical care examination in the third trimester of pregnancy. Then these pregnant women will follow-up until delivery. All patients with late onset preeclampsia (LOPE) will also hospitalize and terminate. The control groups' samples will obtaine during the routine obstetrical care examination in the third trimester of pregnancy. Then these pregnant women were follow-up until the delivery. Both groups will compare 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), thiol, disulphide, and infant weight at delivery. Blood samples were separated by centrifugation for 10 minutes at 1500 *g* after clotting for 30 minutes at room temperature. The serum samples were subsequently stored in aliquots at -80°C prior to the analysis of thiol/disulphide levels. The contents of the native thiol (–SH) and total thiol (–SH + –S–S–) in the serum samples will measure using the reagent kits from Rel Assay Diagnostics (Gaziantep, Turkey). These spectrophotometric methods, developed by Erel and Neselioglu,(1) will assay in an auto-analyzer. The reducible disulfide bonds will first reduce to free-form functional thiol groups. The unused reductant, sodium borohydride, will consume and remove with formaldehyde, and all of the thiol groups, including the reduced and native ones, will measure after reacting with 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB). Half of the difference between the total and native thiols will yield the dynamic disulfide (–S–S) content.

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

The primary endpoint in this analysis is to evaluate thiol/disulfide levels in preeclampsia and address its relationship with its severity.

5.Limitations and strengths:

This study has a limitation that other oxidative parameters such as lipid hydroperoxide, total antioxidant status, total oxidant status and oxidative stress index were not measured.

6. Certain Agreements

The authors declare that they have no conflict of interest.

7. Results Point of Contact

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Reference

1.Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin. Biochem.* 2014; **47**: 326-32.