Full study protocol and statistical analysis plan

Official Title of the study:

Maternal serum levels of selected trace elements and heavy metals in pregnancies

complicated with preterm prelabor rupture of membranes

Date of the document:

August 2018.

Contents:

1. Participant Flow

Recruitment Details

This current observational study was conducted at Cengiz Gokcek Women's and Children's Hospital Gaziantep, Turkey at the Department of Obstetrics and Gynecology between dates of August 2018 and March 2019. The experiment was conducted according to the Declaration of Helsinki. All subjects included in the study gave oral and written informed consents. The study population consisted of 55 women with a singleton pregnancy who were diagnosed with pP-ROM between 24+0 and 36+6 weeks of gestation. The control cases were recruited from the healthy pregnant women with a gestational age-matched cohort who admitted for routine obstetric care to our outpatient clinic. Sixty healthy pregnant women who delivered at term were included in the study as the control group. All patients will gave their oral and written informed consent before their inclusion in the study.

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 *

Preterm prelabor rupture of the membranes group = Group 1
Control group= Group 2

2. Baseline Characteristics

Descriptive statistics for continuous variables are represented as mean, standard deviation, minimum and maximum. Categorical variables are represented as number (n) and percentage (%). The Chi-square test was used to assess the relationship between categorical variables. The Student's t-test was used for the comparison of continuous variables. The Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 22.0. IBM Corp. NY, USA) statistical program was used for all statistical calculations. $p < 0.05$ indicated statistical significance.

3. Outcome Measures

Every woman in the study population underwent obstetric ultrasound examinations and a fetal-maternal assesment was carried out by one of the authors. Obstetric anamneses were obtained from all subjects. The demographic data like age, gravidity, parity, body mass index (BMI) and gestational age were recorded. The protocol for patients with pP-ROM in our hospital was as follows: All patients with pP-ROM were hospitalized and the expectant protocol was applied. After hospitalization until the delivery of the baby, all pregnant women with pP-ROM received prophylactic antibiotics (Ampicillin 1 gr four times a day) for one week and betamethasone injections for lung maturation before 34 weeks of pregnancy (two dosage of 12 mg at 24 h intervals) and magnesium sulfate if delivery was imminent before 32 weeks. The non-stress test and fetal movement determined by the mother were used for detection of fetal well-being. The signs for clinical chorioamnionitis like uterine tenderness, fever, purulent discharges from cervical canal and inflammatory markers like white blood cell count (WBC) and C-reactive protein (CRP) levels were monitored carefully during the hospitalization.

The healthy subjects who had a normal pregnancy period and outcomes without any fetal-neonatal complications were accepted as the control group. All healthy subjects who served as controls were followed up until delivery. The control group's samples were obtained during the routine obstetrical care examination in the third trimester of the pregnancy. Maternal venous blood samples were taken for measurement of selected TEs and HMs levels following the diagnosis of pP-ROM in the outpatient clinic. These samples were quickly centrifuged at 1,500 g for 10 min, plasma and serum samples were separated, and serum samples were stored at -20°C until the day of measurement. The maternal serum levels of trace elements and heavy metals in both groups were measured using an inductively coupled plasma-mass spectrometry (ICP-MS) and compared.

4. *Endpoints of the study:*

The primary endpoint in this analysis is to evaluate maternal serum trace elements and heavy metals namely, aluminum (Al), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), molybdenum (Mo), cadmium (Cd), tin (Sn), antimony (Sb), mercury (Hg), and lead (Pb) in pregnant women complicated by preterm prelabor rupture of the membranes (pP-ROM) and to compare the results with healthy pregnancies.

5.Limitations and strengths:

The main limitation of our study is the small number of patients. As for the strengths of the study, none of the patients had any treatments for preterm prelabor rupture of the membranes and no active labor.

6. Certain Agreements
The authors declare that they have no conflict of interest.

7. Results Point of Contact

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