Full study protocol and statistical analysis plan

Official Title of the study:

Analyses of maternal plasma cadmium, lead and vanadium levels in the diagnosis and severity of late-onset preeclampsia; A prospective and comparative study from Turkey

Date of the document:

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Contents:

1. Participant Flow

Recruitment Details

This observational case-control study was conducted at the Department of Obstetrics and Gynecology, Cengiz Gokcek Public Hospital, Gaziantep, Turkey, between March 2018 and June 2019. The protocol was approved by the Ethics Committee for Clinical Research of Gaziantep University (reference no: 2019/36). 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 46 late-onset preeclampsia patients as study group and 46 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

To detect significant difference between groups according to lead levels with a moderate effect size (Cohen's d =0.62), minimum required sample size was estimated as 42 for each group (α=0.05, 1-β=0.80). Power analysis was performed by using G power package version 3.1. Kolmogorov Smirnov and Shapiro Wilk tests were used to test the normal distribution of data. For comparing groups (L-PrE/control) the student t-test was used for variables that have a normal distribution, and the Mann Whitney U test was used for variables that have not a normal distribution. The ROC analysis was applied for the determination of cut-off point for vanadium variable. Moreover, Spearman correlation test was used for the relationship of between variables. SPSS for Windows 22.0 and 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 plasma samples were taken for measurement of cadmium, lead and vanadium 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, urea, uric acid, albümin, blood urea nitrogen, creatinine, liver function tests (AST, ALT), lactic acid dehydrogenase, cadmium, lead and vanadium concentrions and infant weight at delivery. 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 samples were transferred within boxes in ice molds to Yozgat Bozok University Science and Technology Application and Research Center (Occupational and Environmental Toxicology Laboratory) for measuring heavy metal and trace element levels. The frequency of seafood consumption was divided into four categories: 2-3 per week, 1-2 per week, 1-2 per month, and rare. Smoking was classified into 3 groups (none, formerly smoker, and active smoking). Passive smoking was also categorized into 2 groups (no and yes). The education levels was classified into 4 groups (illiterate,

primary education, high school, and university). The residences of women was classified as rural and urban. Furthermore, the residences of women have divided either living near a busy street (around 100 metres) or not.

Venous blood sampled from the antecubital veins for measuring plasma concentration of cadmium, lead and vanadium levels. The three metals (cadmium, lead and vanadium) were measured using inductively coupled plasma-mass spectrometry (Thermo Scientific ICAPQc, USA).

4. *Endpoints of the study:*

The primary outcome in these analyses was to compare cadmium, lead and vanadium levels in L-PrE group and control group.

The secondary outcome was to compare the cadmium, lead and vanadium levels in mild L-PrE group and severity L-PrE group.

Tertiary outcome was to compare the cadmium, lead and vanadium levels in SGA group and non-SGA group.

5.Limitations and strengths:

Our study has some limitations. Firstly, none of these patients had ever been occupationally exposed to these three metals. Further, the city in which the study was performed is far from the coast. Therefore, the participants' seafood consumption was very low or absent because of the low socio-economic level. Furthermore, our population in this study are more illiterate than others. Because of these reasons, the sample size of the population may not reflect the population in general. Second, the blood samples in the study were only collected at a single time point which may reflect the three metals exposure levels only recent exposure, not before and during pregnancy, and also limiting the ability to establish temporality. Third, it could be even better to measure urinary levels of the metals. Lastly, because our study is observational, we could not eliminate the possibility of unmeasured and/or residual confounding of the reported associations. On the contrary, some confounding factors which are considered as risk factors for PrE and metals exposure are either nearly equally distributed or absent in our patient population. For example, patients with pre-pregnancy systemic diseases, advanced maternal age, multifetal gestation were excluded. Nulliparity, increased body mass index and sociodemographic characteristics such as smoking and traffic exposure of the two groups are similiar.

6. Certain Agreements

The authors declare that they have no conflict of interest.

7. Results Point of Contact

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