Full study protocol and statistical analysis plan

Official Title of the study:

Maternal plasma/urine/hair/amniotic fluid levels of selected trace elements and heavy metals in pregnancies complicated with neural tube defects

Date of the document:

August 2020.

Contents:

1. Participant Flow

Recruitment Details

This observational case-control study will be conducted at the Department of Obstetrics and Gynecology, Cengiz Gokcek Public Hospital, Gaziantep, Turkey, between August 2020 and March 2021. The protocol was approved by the Ethics Committee for Clinical Research of Gaziantep University (reference no: 2020/167). The study strictly will be adhered to the principles of the Declaration of Helsinki. All subjects will be included in the study gave oral and written informed consent. 140 women were enrolled in the study in two groups.

Pre-assignment Details

The authors first will be assessed the recruited people to ensure meeting the inclusion and exclusion criteria. The inclusion criteria will be made according to the official documents.

Arm/Group Information *

There are two groups in the study.

Arm/Group Title *

Neural tube defect group

Control group

2. Baseline Characteristics

To detect significant difference between groups according to lead levels with a moderate effect size (Cohen's d =0.53), minimum required sample size was estimated as 45 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 will be used to test the normal distribution of data. For comparing groups (NTD/control) the student t-test will be used for variables that have a normal distribution, and the Mann Whitney U test will be used for variables that have not a normal distribution. The ROC analysis will be applied for the determination of cut-off point for variables. Moreover, Spearman correlation test will be used for the relationship of between variables. SPSS for Windows 22.0 and Medcalc programs will be used for statistical analysis. $p<0.05$ will be accepted as statistical significance.

3. Outcome Measures

Primary Outcome Measures : folic acid, zinc (Zn), molybdenum (Mo), vanadium (V), strontium (Sr), aluminum (Al), tin (Sn), antimony (Sb), mercury (Hg), calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), barium (B) and selenium (Se) concentrations

Secondary Outcome Measures:

The elements and vitamins concentrations in the spinal NTD group and the cranial NTD group.

4. *Endpoints of the study:*

The primary outcome in these analyses will compare folic acid, zinc (Zn), molybdenum (Mo), vanadium (V), strontium (Sr), aluminum (Al), tin (Sn), antimony (Sb), mercury (Hg), calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), barium (B) and selenium (Se) concentrations in NTD group and control group.

5.Limitations and strengths:

Our study has some strengths. These metals will be measured blood, urine and hair samples. So, these measurements may not reflect the metals exposure levels only recent exposure and will reflect before and during pregnancy.

6. Certain Agreements

This work will be supported by the Scientific Research Project Fund of Yozgat Bozok University.

7. Results Point of Contact

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