Full study protocol and statistical analysis plan

Official Title of the study:

Endothelial cell-specific-molecule-1 (endocan) levels in women with premature ovarian insufficiency

Date of the document:

July 2018.

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 July and December 2018. Seventy-seven women were enrolled in the study in two groups. We consecutively recruited 38 subjects with idiopathic POI, and 39 healthy patients were selected for the control group. All patients 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 *

POI group = Group A
Control group= Group B

2. Baseline Characteristics

In two groups (poi - control), the total number of individuals required to find a statistically significant effect size expectation was calculated as 72 (α=0.05, 1-β=0,80) in terms of endocan measurements. The normality of distribution of continuous variables was tested using the Shapiro-Wilk test. To compare numerical variables between 2 groups, Student's t-test (for normal data) or the Mann-Whitney U test (for non-normal data) was performed. The Chi-square test was used to assess the relationship between categorical variables, and Spearman's rank correlation coefficients were used to assess the relationship between non-normal numeric data. Frequency, percentage (%) and mean ± standard deviations (mean ± SD) are given as descriptive statistics. Statistical analysis was performed using the SPSS for Windows version 24.0 software package, and p valued < 0.05 were accepted as statistically significant.

3. Outcome Measures

At enrollment, for both groups, we collected data about age, height, weight, BMI, age of menarche, obstetrics history, history of smoking, regular exercise and family history of POI. We defined that POI period is a time from diagnosis to admission. At enrolment, all patients underwent vaginal ultrasonography for the assessment of antral follicle count (AFC) and venous blood sample from the antecubital veins for measuring serum concentration of Endocan, Follicle-stimulating hormone (FSH), E2, anti-mullerian hormone (AMH) and complete blood count (CBC). In control subjects venous blood samples and AFC were collected during the early follicular phase of the menstrual cycle (2nd to 5th days) in the morning (between 08.00 and 09.00 h). In the POI group, measurements were repeated with 4-week intervals. AMH was not measured in the control group. AFC were assessed through vaginal ultrasonography by the same author. 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 endocan. The serum endocan level was

measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, which is produced to detect human endocan levels with high sensitivity and specificity. The endocan measurements were performed in accordance with company's protocol. The kit uses the sandwich ELISA principle. A biotinylated detection antibody specific for human ESM1 (endocan) and avidin-horseradish peroxidase conjugate were used in the measurement. Spectrophotometry at a wave length of 450 ± 2 nm was used in the detection of optical density, which is proportional to the concentration of human endocan level. The intra- and inter-assay variation coefficients were 6.36% and 6.09%, respectively.

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

The primary endpoint in this analysis was to compare endocan levels in POI group and control group. The secondary endpoint was to compare endocan levels in POI group for POI period. Tertiary endpoint was to compare the endocan levels in both groups according to the births.

5.Limitations and strengths:

There are strengths of this study. The women participating in the study had idiopathic POI without any treatment. The women who have not taken any hormonal treatments, alternative and complementary treatments that may have possible potential effects on endocan levels.

6. Certain Agreements

The authors declare that they have no conflict of interest.

7. Results Point of Contact

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