Comparison of Serum C Type Natriuretic Peptide Levels Between Polycystic Ovary Syndrome Patients and Healthy Women
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Study Protocol and Statistical Analyses Plan

Recent studies have shown that C natriuretic peptide (CNP) is produced from granulosa cells, increasing cumulative guanosine monophosphate (cGMP) production by affecting cumulus cells through natriuretic peptide receptors. It is suggested that produced cGMP maintains the transport of oocytes via the gap junctions and leads to a continuous increase in cyclic adenosine monophosphate (cAMP) levels in the oocyte. An important role of increased internal cAMP levels in the oocyte is shown to suppress meiotic progression.

Deoxyribonucleic acid studies in animals have shown that expression of the natriuretic peptide precursor increases during the periovulatory period and shows that this increase decreases rapidly after luteinizing hormone (LH) / human chorionic gonadotropin (hCG) stimulation. Human studies have shown that after ovulation induction, the CNP level in follicular fluid decreases following ovulatory dose of hCG.Polycystic ovary syndrome (PCOS) is the most common endocrine disease in the reproductive period, characterized by hyperandrogenism, oligo-anovulation, and polycystic ovarian morphology on ultrasonography. Recently, a study on PCOS-like mouse model investigated the effect of CNP/ natriuretic peptide receptor 2 (NPR2) on oocyte maturation and anovulation. They increased the expression of CNP/NPR2 with hyperandrogenism and showed meiotic arrest and anovulation in the subject oocytes. Conversely, they showed that reducing CNP/NPR2 production led to partial recovery of ovulation.CNP serum level is thought to show differences between healthy women and women with polycystic ovary syndrome.

In the light of these data, we conclude that in PCOS, which is known to be associated with hyperandrogenism and anovulation, serum levels of CNP, which is also associated with these components, may be elevated. In this study, it was aimed to determine serum CNP levels in PCOS patients, their relationship with serum androgens, and comparison with the healthy group.

In this prospective study, a total of 66 patients admitted to the Department of Obstetrics and Gynecology of the Near East University Hospital were included. PCOS group consisted of 36 women and control group included 30 healthy women with regular menstruation aged between 18-40 years old. PCOS diagnosis was made according to Rotterdam criteria. PCOS diagnosis was made in the presence of two or three of these findings; oligo/anovulation, polycystic ovaries on ultrasonography and clinical or biochemical manifestations of hyperandrogenism.

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Age, body mass index (BMI), serum levels of LH, follicle stimulating hormone (FSH), estradiol, thyroid stimulating hormone (TSH), prolactin (PRL), androstenedione, dehydroepiandrosterone sulfate (DHEAS), total testosterone, free testosterone, sex hormone binding globulin (SHBG), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), glucose and insulin levels were measured. For insulin sensitivity, homeostatic model of insulin resistance (HOMA-IR) was used. Free androgen index (FAI) was calculated for all participants.

Exclusion criteria were as follows: age over 40 years, current pregnancy, hyperprolactinemia, thyroid disease, congenital adrenal hyperplasia, a history of ovarian surgery or any other systemic disease or drugs that could influence hypothalamic pituitary ovarian axis, androgen production, insulin and or/glycemic metabolism, patients with cardiac or renal disease and therefore drug use, patients who received any hormone therapy including contraceptives up to 6 months before study.

Morning fasting venous blood samples were taken from the patients on the 2nd or 3rd day of the menstruation for all participants. All blood samples were centrifuged on the day of collection and separated serum samples and were kept at -80 degrees celcius until the day of CNP test. Serum CNP levels of the patients were analyzed by an enzyme-linked immunosorbent (ELISA) assay for human CNP in accordance with the manufacturer's instructions (SEA721Hu, ELISA Kit for Human CNP, Wuhan USCN Business Co., Ltd., Cloud-Clone Corp., CCC, USA).

Data were analyzed using Statistical Package for Social Sciences software (SPSS v15, SPSS Inc, Chicago, IL, USA). Data were presented as mean±standard deviation or median and interquartile range. Independent t-test was used to compare the parameters with normal distribution. Parameters that don’t fulfill the parametric test assumptions were compared using Mann-Whitney U test. Correlation of CNP with other parameters was analyzed using Spearman’s rank correlation test. Receiver operating characteristic (ROC) curve was used to evaluate diagnostic sensitivity and specificity of CNP for PCOS. P values less than 0.05 were regarded as statistically significant.