Serum Levels of C Type Natriuretic Peptide in Different Reproductive Periods

NCT04000815

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Study Protocol and Statistical Analyses Plan

Recent studies have shown that C type natriuretic peptide (CNP) that is produced from granulosa cells of the ovary, has an increasing effect on cyclic guanosine monophosphate (cGMP) production by affecting cumulus cells through natriuretic peptide receptors (NPR). It has been suggested that the transport of cGMP to oocyte via gap junctions causes an inhibition on oocyte phosphodiesterase 3 which leads an increase in cyclic adenosine monophosphate (cAMP) levels within the oocyte by hydrolyses. An important role of increased cAMP levels in oocyte is shown to suppress meiotic progression. Hormonal regulation of CNP secretion has been demonstrated in animal and human studies. Deoxyribonucleic acid (DNA) studies in animals have shown that expression of the natriuretic peptide precursor (NPPC) increases during the periovulatory period and shows that this increase decreases rapidly after Luteinizing hormone(LH) / human chorionic(hCG) stimulation. Human studies have shown that after ovulation induction, the CNP level in follicular fluid decreases following ovulatory dose of hCG. It has been demonstrated by many studies that LH has a suppressing effect on NPPC / NPR2 production. In other studies, esradiol has been shown to have a promoting effect on NPPC. Follicle stimulating hormone (FSH) alone, has been shown to have no effect on CNP regulation. In fact when there is estrogenic effect together with FSH, there is increasing CNP levels.

C type natriuretic peptide, which has been demonstrated that is affected by E2, LH and FSH in the hormonal regulation and is synthesized by the granulosa cells, may be a new ovarian reserve test. And also, it may be associated with increased age, which is also an important factor in ovarian aging. In this study, it was aimed to determine and to compare serum CNP values in three different stages of reproductive life.

In this prospective study, 71 patients are recruited. After the exclusion of patients, group 1 consists of 15 healthy reproductive aged women between 18-40 years old, with regular menstruation. Group 2 includes 15 patients in perimenopausal time period between 40-49 ages and in group 3 there is 15 postmenopausal women.

Age, gravida, parity and body mass index (BMI) data of all patients are recorded. BMI is calculated by dividing the body weight in kilograms by the square of the height in meters. All patients went under ultrasound examination by the same clinician (ACO). The number of antral follicles in group 1 and group 2 were recorded. In addition, on the 2nd or 3rd day of menstruation, serum FSH, LH and E2 data of the patients in group 1 and 2 were recorded.

Patients with drug use that may affect menstruation like oral contraceptives in the last 6 months, patients with a known cardiac or renal disease and therefore using medicine, patients

suffering from infertility as a result of any reason other than unexplained etiology, patients with a history of ovarian surgery, presence of polycystic ovary syndrome or that have an irregular menstrual cycle were excluded from this study. In addition, patients with central nervous system and endocrine diseases were excluded.

Morning fasting venous blood samples were taken from the patients on the 2nd or 3rd day of the menstruation for group 1 and 2. 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). The variables were investigated using visual and analytical methods (histograms, homogeneity of variances test, Kolmogorov-Simirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. Patient demographics and CNP values were presented as median and interquartile range. Gravida and parity were demonstrated by frequency distribution. The correlation coefficients and their significance were calculated using the Spearman test. P-values less than 0.05 will be regarded as statistically significant.

Results

A total of 45 patiens were evaluated in the statistics. The flow chart of the study is shown in figure 1. Table 1 shows the demographic characteristics and table 2 shows frequency distribution of gravida and parity of the patients. In table 3 serum CNP levels are shown. The median and interquartile range of serum CNP levels in groups 1, 2 and 3 were, 43.68 (35.54-47.87) pg/mL, 55.98(49.84-61.13) pg/mL and 116.36(59.61-250.00) pg/mL, respectively. When the three groups were compared with Kruskal Wallis test, there was a significant difference between them in terms of serum CNP values ($p<0.001$). Post hoc analysis showed significant differences between group 1 vs. group 2, group 1 vs. group 3 and group 2 vs. group 3. (p values; $p <0.001$, $p <0.001$, and $p = 0.002$, respectively). When all patients were evaluated, a positive correlation was found between age and serum CNP levels. ($R_s= 0.712$, $p<0.001$) (Figure 2) In group 1 and group 2, there was a positive correlation between serum CNP and serum FSH, ($R_s=0.609$, $p<0.001$) (Figure 3) and a negative correlation between serum CNP and AFC. ($R_s= -0.603$, $p<0.001$) (Figure 4) There was no correlation between serum CNP and serum LH and E2 values in group 1 and 2.

Figure 1: Flow chart of the study

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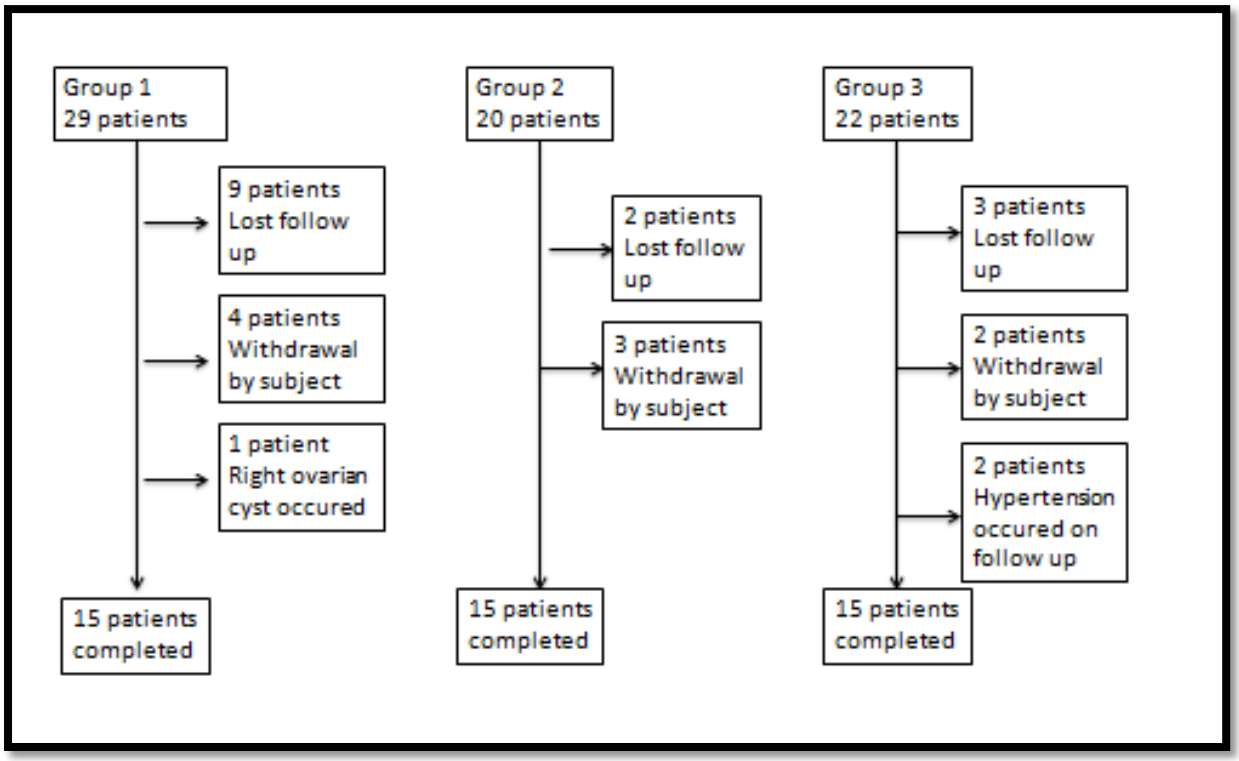

Table 1. Demographic characteristics of participants

	Group 1(n=15)	Group 2 (n=15)	Group 3 (n=15)	All participants (n=45)
Age (years)	24.9±2.9	44.1±2.2	57.7±5.4	42.2±14.1
BMI (kg/m^2)	21.3±1.9	24.3±2.4	27.1±4.1	24.2±3.8
AFC	14.2±2.18	8.33±1.72	-	11±7
FSH (mIU/mL)	5.28±1.33	13.12±7.3	-	7.65±7.96
LH(mIU/mL)	5.71±2.51	5.16±2.45	-	5.29±2.76
E2(pg/mL)	41±26	22±14	-	32±27

BMI: Body mass index, AFC: Antral follicle count, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol

Table 2: Frequency distribution of gravida and parity among groups

Frequency	Group 1 (n=15)		Group 2 (n=15)		Group 3 (n=15)	
	Gravida	Parity	Gravida	Parity	Gravida	Parity

0	14	14	1	1		
1	1	1	1	4		1
2			8	8	5	10
3			4	2	4	2
4			1		3	1
5					1	
7						1
10					2	

Table 3. Serum CNP levels of participants

	Group 1(n=15)	Group (n=15)	Group 3 (n=15)	p
CNP (pg/mL)	43.68 (35.54-47.87)	55.98(49.84-61.13)	116.36(59.61-250.00)	**<0.001**

CNP:C type natriuretic peptide

Group 1vs group 2: **p<0.001**; group 1 vs group 3: **p<0.001**; group 2 vs group 3: **p=0.002**.

Figure 2.Correlation between age and serum CNP

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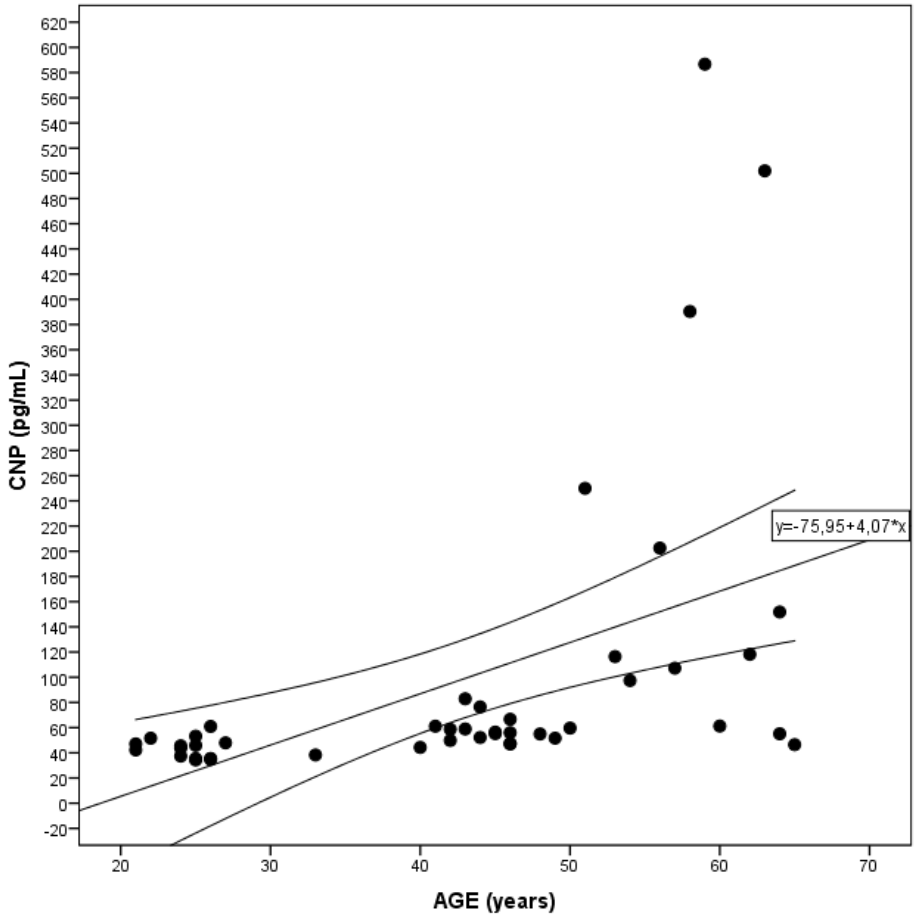

Figure 3.Correlation between serum CNP and serum FSH

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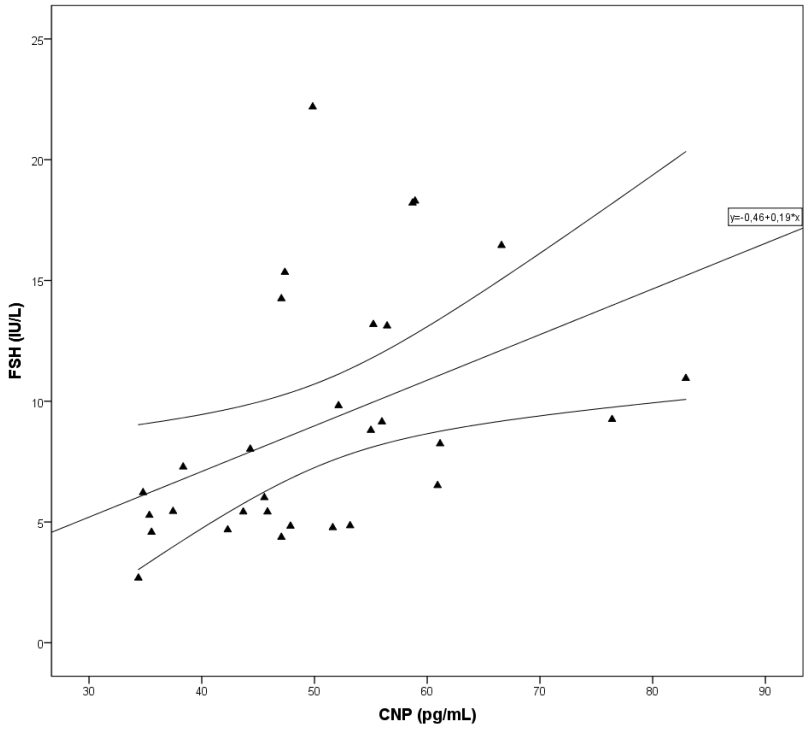

Figure 4.Correlation between serum CNP and AFC