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Original Article

# Comparison of serum c-type natriuretic peptide levels in different genders: A pilot study

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ABSTRACT

Objective: Firstly we aimed to compare serum C-type natriuretic peptide (CNP) levels in healthy male and female participants of similar age group and secondly to reveal the relationship between serum CNP values and ovarian function markers in women and sperm parameters in men.

Materials and Methods: In this prospective study, a total of 40 patients, who were admitted to the Department of Obstetrics and Gynecology at Near East University Hospital were included. Of these, 20 were female and 20 were male participants. Serum CNP levels of the patients were analyzed for all participants. Day 3 serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) for female participants and semen samples after 3-5 days of sexual abstinence for male participants were evaluated.

Results: Serum CNP levels were similar in both genders. There was only a moderate, but statistically insignificant negative correlation between serum CNP and FSH in female group. In the correlation analysis performed in the male group, no significant correlation was observed between serum CNP level and any semen analysis parameter.

Conclusion: We showed that serum CNP levels were similar between healthy women and men. There was no relationship between levels of serum CNP and reproductive hormones in the early follicular phase of menstruation in women, and semen parameters in men.

Keywords: C-type natriuretic peptide; genders; semen analyze

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## Introduction

In 1990, C-type natriuretic peptide (CNP), which belongs to the family of natriuretic peptides was shown for the first time that was produced in the porcine brain.[1] Unlike other natriuretic peptide family members, CNP is produced mainly from extracardiac tissues and acts as a local autocrine/paracrine regulator. [2] Studies are showing that it is synthesized in many organs including male and female reproductive systems. [3-7] CNP acts by binding to natriuretic peptide receptor B (NPR-B). [2,8] Through the CNP / NPR-B pathway, a series of reactions occur in reproductive organs, including the intracellular secondary messenger cyclic guanosine monophosphate (cGMP), and important functions such as meiotic progression are affected. [6,8] And also, it is stated in the studies that CNP has a role that has not been clearly defined yet on the hypothalamo-pituitary-gonadal axis. [9,10]In the process of gametogenesis, a large number of factors are effective in the hypothalamo-pituitary-gonadal axis and any disruption in this pathway may be the cause of infertility in both sexes.

Gonadotropin releasing hormone, gonadotropins, and estradiol affect hormonal regulation of CNP and this effect has been studied and demonstrated in the female reproductive system. [11-13] Many studies have shown that CNP is effective in the female reproductive system. [6-8] The most obvious of these effects is the meiotic arresting effect of CNP. [6,8] CNP production has been demonstrated in the uterus and ovaries, and it has been shown to increase dose-dependent in the uterus to estradiol. [14] In a study conducted in 1997, it was stated that the proestrus CNP production was at the highest level. [15,16] Tamura et al. showed that NPR-B null mice had (NPR-B -/-) female sterility and small uterus and ovaries. [17]

The number of studies on the effect of CNP on the male reproductive system is less when compared to studies on the female reproductive system. Total count, motility, and morphologic characteristics of sperm are the most used and important parameters that affect male fertility. CNP production was found to be at the highest concentrations in male reproductive organs and it may affect male fertility.

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[5,18] In a study in 2003, CNP was shown to play an important role in male fertility, including endocrine functions in the testicle and penile erection. [19]
The effect of CNP on the regulation of the dynamics of the blood testicle barrier during the spermatogenesis process has been demonstrated in rats. [20] Besides, another study suggested that there is a relationship between N-terminal proCNP (NTproCNP, which is the precursor of CNP) and asthenozoospermia. [21] And in a recent study, it has been demonstrated that sperm motility and acrosome reaction are also affected by CNP levels in the environment. [22]
In this study, it was aimed to compare serum CNP levels in healthy male and female participants of similar age group. The second aim of this study is to reveal the relationship between serum CNP values and ovarian function markers in women and sperm parameters in men.

## Material and methods

This study was carried out between 1 November 2019 and 15 February 2020 at the Near East University Faculty of Medicine, Department of Obstetrics and Gynecology. Ethics committee approval was obtained from the local ethics committee and all participants signed the informed consent before enrollment to the study (YDU/2019/75-948).
In this prospective study, a total of 40 patients, who were admitted to the Department of Obstetrics and Gynecology at Near East University Hospital were included. Of these, 20 were female and 20 were male participants.
All participants were evaluated with a detailed history and physical examination, electrocardiogram, and renal function tests. Age, body mass index (BMI), renal and cardiac disease states of all participants were recorded. BMI was calculated by dividing the body weight in kilograms by the square of the height in meters. All female participants underwent ultrasound examination by the same clinician (ACO). Day 3 serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) were recorded.
Male participants gave semen samples after 3-5 days of sexual abstinence. Sperm analyzes were done conventionally and evaluated according to World Health Organization guidelines. Leica DM1000 microscope (Leica, Mannheim, Germany) was used for sperm evaluation. Volume, liquefaction, viscosity, sperm count, motility, progressive motility, peroxidase positive leukocyte, and Kruger's morphology were evaluated in all sperm analyzes.
Fasting venous blood samples were taken and centrifuged in the morning on the day the sperm sample was taken for male participants and on the 2nd or 3rd day of menstruation for female participants. Serum samples were kept at -80 °C until CNP was studied. Serum CNP levels of the patients were analyzed by an enzyme-linked immunosorbent (ELISA) assay for human CNP following the manufacturer's instructions (SEA721Hu, ELISA Kit for Human CNP, Wuhan USCN Business Co., Ltd., Cloud-Clone Corp., CCC, USA).
The patients with systemic disease that was previously diagnosed or detected in our examinations and tests (cardiac, renal, central nervous system, hyperlipidemia, and endocrine diseases), any cause for infertility, history of ovarian or testicular surgery were excluded. Also, the men with erection problems were not included in the study. Besides, for female patients, drug usage for the last 6 months that may affect menstruation like oral contraceptives, presence of polycystic ovary syndrome, irregular menstruation were exclusion criteria for the study.

*Statistical analysis*
The Statistical Package for the Social Sciences (SPSS) v.15 for Windows (SPSS Inc., Chicago, U.S.A.) was used for all the statistical analyses. Age, BMI, and CNP values were reported as median values and interquartile range. The comparisons between the groups were performed by the Mann-Whitney U test. Correlation analyses were carried out using Spearman's rank. A difference was regarded as statistically significant if the P-value was smaller than 0.05.

## Results

A total of 40 patients were evaluated in the statistics. When the male and female groups were evaluated, serum CNP and ages of participants were found to be similar. Although statistically insignificant, the median BMI value was higher in the male group (Table 1).

Table 1. Demographic characteristics and serum C-type natriuretic peptide values of the participants

| | Female (n=20) | Male (n=20) | p |
|---|---|---|---|
| Age | 23.0 (3.5) | 23.0 (2.5) | 0.529 |
| BMI (kg/m$^2$) | 21.95 (2.56) | 23.90 (2.78) | 0.052 |
| CNP (pg/mL) | 80.94 (107.51) | 118.49 (62.25) | 0.341 |

In the female group, a correlation test was performed between serum CNP level and reproductive hormones. There was only a moderate, but statistically insignificant negative correlation between serum CNP and FSH (Table 2).

Table 2. Correlations of serum C-type natriuretic peptide with hormonal parameters of the female group

| | FSH | LH | Estradiol |
|---|---|---|---|
| Correlation Coefficient | -0.385 | -0.134 | -0.037 |
| P | 0.094 | 0.574 | 0.877 |

*FSH: Follicle stimulating hormone, LH: Luteinizing hormone*

In the correlation analysis performed in the male group, no significant correlation was observed between serum CNP level and any semen analysis parameter (Table 3).

Table 3. Correlations of serum C-type natriuretic peptide with sperm parameters of male group

| | Volume | Concentration | Total count | Total motility | Progressive motility |
|---|---|---|---|---|---|
| Correlation Coefficient | -0.183 | 0.298 | 0.084 | 0.050 | 0.127 |
| P | 0.439 | 0.202 | 0.724 | 0.835 | 0.595 |

## Discussion

After the demonstration of the production of CNP especially from reproductive organs and its association with reproductive hormones, publications in this direction have increased. In our study, serum CNP levels did not differ significantly in two healthy groups considered as male and female of similar ages.
It has been shown in the literature that CNP is associated with body mass index in adolescents. [23] It was also stated in this study that serum CNP levels change with age. [23] Therefore, in our study, the groups were taken as similar in terms of age and BMI. In addition, due to the small number of participants in our study, BMI subgroups were not formed and evaluated.
In this study, after excluding additional pathologies such as renal and cardiac diseases that may affect serum CNP levels, we compared it for both genders and we did not observe a statistically significant difference. Prickett et al found that serum NTproCNP values were statistically significantly higher in male participants, but they did not find a significant difference in serum CNP values between genders, similar to our study results. [24] This may be due to differences in the half-life or metabolism of NTproCNP and CNP in serum.

In the literature, there are studies that stated reproductive hormones are playing a role in the secretion of CNP. [25,26] Kawamura et al. showed in their study that LH reduces CNP production in follicular fluid in the human oocyte. [25] On the other hand, Lee et al. stated that there was an increase in CNP production after E2 alone, E2 plus LH, or E2 + LH + FSH. [26] In our study, serum reproductive hormones and CNP levels were examined in women in the early follicular phase and there was no statistically significant correlation between serum CNP levels versus E2, LH, and FSH. This may be due to several reasons. First of all, because our study was a pilot study, the number of patients was limited. Secondly, the secretion of reproductive hormones changes throughout the menstrual cycle, and since we only evaluated the early follicular period, it would not be correct to comment on the entire menstrual cycle with this study. Furthermore, the concentrations in the circulation may not reflect local activities, receptor status, and paracrine and autocrine effects of hormones.

CNP is secreted in the reproductive organs in men as well as in women and it has been shown that CNP affects many parameters related to reproductive functions in men. Tomasiuk et al., compared the asthenozoospermic and normal groups in their study and they found that NTproCNP levels in ejaculate were significantly higher in the asthenozoospermic group. [21] Additionally, Xia et al. showed that CNP increases sperm motility in normozoospermic participants. [22] In our study, differently, we examined the relationship between serum CNP levels and semen parameters in healthy normozoospermic men and we found no significant correlation between serum CNP and any semen parameters. In fact, the reason why we did not find any correlation may be the small number of participants. In their study, Vlachopoulos et al found that serum CNP levels were associated with presence, severity, and duration of erectile dysfunction. [27] All of the male participants in our study consisted of healthy individuals in terms of sperm parameters. Although it was not among the aims of our study, none of the participants reported any erectile dysfunction.

Although designed as a pilot study, the most important limitation is the small number of participants. The value of this study shall increase with a higher number of participants, the evaluation of patients with different BMI values, the inclusion of patients with pathological semen analysis results and erectile dysfunction in male patients, and analyzing serum CNP samples taken at different times of the reproductive period in female patients.

CONCLUSION

In conclusion, in this study, we showed that serum CNP levels were similar between healthy women and men. Also, we found no relationship between serum CNP and reproductive hormones in the early follicular phase of menstruation in women, and with semen parameters in men. Considering the limitations of our study and that it is a pilot study, further studies are needed to confirm the results we have produced.

## Disclosure

Authors have no potential conflicts of interest to disclose.

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