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Prokineticin 1 – Is it a reliable biomarker in polycystic ovarian syndrome?

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Abstract. – OBJECTIVE: Polycystic ovarian syndrome (PCOS) is a very common endocrine disorder that leads to follicular dysfunction and even infertility. Prokineticin 1 (PROK1) has been shown to be released from the theca interna and stroma of ovaries. PROK1 is also known to be a major prokineticin involved in reproduction and to play a crucial role in ovarian physiology. The present study investigated whether PROK1 might be a reliable biomarker for PCOS.

PATIENTS AND METHODS: **The present study was conducted in Koru Ankara Hospital, Department of Obstetrics and Gynecology. Of the 88 women included in the study, 44 were diagnosed with PCOS. The remaining comprised the control group. Ten ml of venous blood sample was taken from every woman and stored at -80°C until analysis. All tests were two-tailed, and a *p*-value <0.05 was considered statistically significant.**

RESULTS: **There was no significant difference between the groups in terms of age. The mean serum levels of LH/FSH and E2 were significantly higher in PCOS patients compared to controls. No statistically significant difference was found in PROK1 levels between the groups.**

CONCLUSIONS: **PCOS is a very common endocrine disease affecting the life quality of a woman from adolescence to reproductive age. Some *in vitro* animal studies and a few human studies have revealed higher PROK1 levels in women with PCOS compared to healthy controls. To the best of our knowledge, this is the first in vivo study on humans; investigating the relationship between PROK1 and PCOS. However, larger-scale studies may be designed to reveal the role of this peptide.**

Key Words:
Angiogenesis, Biomarker, MicroRNA, Prokineticin 1, Polycystic ovarian syndrome.

Introduction

Prokineticins are a group of peptides involved in angiogenesis[1] and reproduction[2,3]. Moreover, they have been reported to have an important role in hematopoiesis[2], neuronal activity[3] and immune response[2]. They are basically classified into three groups: PROK1 (prokineticin 1), PROK2 and PK2L (longer form of PROK2)[1]. PROK1, a peptide consisting of 86 amino acids, has been reported to be a major prokineticin involved in many vital steps of female reproductive processes, especially ovarian physiology, endometrial receptivity, and even embryo implantation[4]. Owing to its key role in angiogenesis[4], it is also named as endocrine gland-derived vascular endothelial growth factor (EG-VEGF).

Polycystic ovarian syndrome (PCOS) is one of the most common female endocrine disorders of unknown pathogenesis. It is diagnosed in 10-25% of women of reproductive age[5]. The diagnostic features of the disease include oligo-anovulation, polycystic ovarian morphology visualized on ultrasound, and findings of hyperandrogenism[6]. High levels of androgens which impair follicular functions result in irregular menstrual periods and even infertility[7]. In PCOS, strong expression of PROK1 mRNA has been demonstrated in the theca interna and stroma of ovaries[8]. Revealing the association of PROK1 with PCOS requires further investigation. Therefore, the present study was conducted to clarify whether PROK1 could be used as a biomarker of PCOS.

Patients and Methods

This prospective study was conducted in Koru Ankara Hospital, Department of Obstetrics and Gynecology, between April 2021 and September 2021. A total of 88 women were included in the study. Forty-four of them were diagnosed with PCOS, while the remaining 44 healthy women comprised the control group.

All participants underwent a routine assessment, including a detailed history, body mass index (BMI) measurement, day 1-3 follicle stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E2) (following a spontaneous or pro-

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gesterone-induced bleeding) serum tests. The antral follicles of the two groups were counted using transvaginal ultrasound. Patients were diagnosed with PCOS according to the Rotterdam criteria[6]. A menstrual cycle was considered oligo-anovulation in the case of a menstrual cycle length of greater than 35 days. Polycystic ovarian morphologic features are defined as at least one ovary with 12 or more follicles between 2 and 9 mm in diameter. All participants were considered eligible unless they were not on active hormonal therapy.

Those with chronic hypertension, type 1 diabetes mellitus or morbidly obese individuals were excluded from the study. Furthermore, smokers and women who had undergone any surgical intervention were excluded. The study protocol was approved by the Ethical Committee of Ankara City Hospital (E2-22-1870). All the participants provided written informed consent. About 10 ml of venous blood sample was taken from each participant and dispensed into lithium heparin. After obtaining serum, then they were stored at -80°C until analysis. The demographic characteristics of the participants were recorded. Serum PROK1 measurements were performed using an enzyme-linked immunosorbent assay (ELISA) kit (Hangzhou Eastbiopharm, Hangzhou).

Statistical Analysis

SPSS, version 25.0-software (IBM Corp., Armonk, NY, USA) was used for data analysis. Descriptive statistics included mean, standard deviation, median and interquartile range (IQR). Levene's test was used to test for homogeneity. The Shapiro-Wilk test was used to check whether continuous data followed a normal distribution. Student's *t*-test was used to analyze the difference between the groups. Non-parametric Mann–Whitney U test was used for non-normally distributed variables. A p-value <0.05 was considered statistically significant.

Results

The study included 44 women with a diagnosis of PCOS as the patient group and 44 age- and BMI-matched healthy women as the control group. The demographic characteristics and hormonal levels of the participants were shown in Table I. There was no significant difference between the groups in terms of age (years) (U=-1.908 p=0.056) (Table I). The groups had statistically significant differences in LH/FSH (U=-7.708 p=0.001), E2 (Estradiol) (U=-2.237 p=0.025), MPV (Mean Platelet Volume) (U=-2.232 p=0.026) and BMI (t=3.680 p=0.001). The mean serum levels of LH/FSH and E2 were significantly higher in PCOS patients than in controls. The mean BMI was significantly higher in the PCOS group than in the control group. The PCOS group, had significantly lower serum levels of MPV compared to the control group.

Prokineticin-1 levels were similar in the study groups with no statistically significant difference (U=0.426 p=0.670) (Table II). This result can be attributed to the small number of participants in both groups.

Table I. Clinical characteristics of the study participants (n=88).

	Polycystic Ovarian Syndrome Mean+/- SD M (Q1; Q3)	Control Mean+/- SD M (Q1; Q3)	Critic Value	p
Age	29.18±4.848 28 (26;31)	30.84±5.103 31 (27;35)	-1.908	0.056
LH/FSH	2.26±1.258 2 (1;2)	0.76±0.23 1 (1;1)	-7.708	0.001**
E2	74±55.969 50 (37;99)	46.06±25.794 39 (33;55)	-2.237	0.025*
MPV	10.04±0.752 10 (9;11)	10.4±0.522 11 (10;11)	-2.232	0.026*
BMI	25±3.596 25 (22;28)	22.34±3.169 22 (20;25)	3.680	0.001**

*p<0.05; **p<0.01 SD: Standard deviation; M: Median; Q_1: 1st quarter; Q_3: 3rd quarter.

Table II. Prokineticin 1 levels of the groups (n=88).

	Polycystic Ovarian Syndrome Mean+/-SD M (Q1; Q3)	Control Mean+/-SD M (Q1; Q3)	Critic value	*p*
Prokineticin 1 Replicate_OD (pg/ml) (mean ±SD)	0.53±0.247 0 (0;1)	0.52±0.15 0 (0;1)	-0.426	0.670
Prokineticin 1 Replicate_Conc	53.11±53.952 44 (23;63)	49.65±29.727 45 (25;72)	-0.426	0.670
Prokineticin 1 Mean_OD	0.53±0.247 0 (0;1)	0.52±0.15 0 (0;1)	-0.426	0.670
Prokineticin 1 Mean_Conc	53.11±53.952 44 (23;63)	49.65±29.727 45 (25;72)	-0.426	0.670

SD: Standard Deviation; M: Median; Q_1: 1st quarter; Q_3: 3rd quarter.

Discussion

PCOS is a common endocrine disorder complicated by several reproductive and metabolic problems. Both genetic and environmental factors are involved in the pathogenesis of PCOS[9]. Follicular growth is activated in every step of ovarian development in PCOS. Cell proliferation and defective oocyte apoptosis are believed to be responsible for the development of the disease[10].

PROK1 is predominantly expressed in the ovary and shows angiogenic effects[4]. Besides, the placenta has been shown to be an important source of PROK1, with its levels being higher in patients with preeclampsia[11]. PROK1 has also been reported to be involved in embryo implantation[12].

Since PROK1 affects endometrial receptivity and embryo implantation, these steps can be easily affected in PCOS. Inositol supplementation is beneficial for implantation, in PCOS; however, injection of embryo culture supernatant to the endometrial cavity has been shown to have no effect on implantation and pregnancy rates in intracytoplasmic sperm injection (ICSI) cycles[13].

Inositol plays multiple roles in PCOS, one of which is its supplementation prior to *in vitro* fertilization (IVF) after failure due to sperm defects like cryptic defects[14]. Even the relationship of PROK1 with the prognosis of epithelial ovarian cancer has been investigated in the literature[15].

Ujvari et al[16] investigated the effect of insulin on PROK1 expression by collecting endometrial stromal cells from six healthy women and decidualizing them in laboratory conditions. They showed that insulin induced a statistically significant increase in the expression of PROK1 mRNA in endometrial stromal cells, which caused an inadequate invasion of trophoblasts in pregnancy. The natural polyol myo-Inositol (myo-Ins) has proven to improve PCOS features and clinical outcomes. Alpha-lipoic acid (ALA) is also known to antagonize insulin resistance. Besides, it has anti-inflammatory and antioxidant activities. Since the dysregulation of the glucose pathway and insulin resistance are involved in the pathogenesis of PCOS, ALA seems to be useful in the treatment of this syndrome. However, a significant positive effect on LH and FSH can be obtained only when myo-Ins is associated with ALA[17].

In PCOS, PROK1 mRNA has been reported to be highly expressed in the theca interna and stroma of ovaries[18]. For this reason, PROK 1 has been accounted for the progression of PCOS. The nature of the factors that mediate angiogenesis in PCOS has not clearly been understood. Ferrara et al[19] reported that PROK1 was strongly associated with hyperplasia and angiogenesis in PCOS.

The study of Agrawal et al[20] showed the vital role of PROK1 in angiogenesis and higher serum levels of PROK1 in women with PCOS compared to healthy controls. Gao et al[21] revealed the value of follicular fluid and serum VEGF concentrations for predicting Ovarian Hyperstimulation Syndrome (OHSS). Moreover, they declared in their study that PROK1 concentrations were much more successful in reflecting OHSS and even its severity. LeCouter et al[4] suggested that PROK1 carried by adenovirus led to an intense angiogenic effect and cyst formation in the ovary whereas it had no effect when carried to other organs like skeletal muscle. MicroRNAs (miRNAs) are small RNAs that impair the expression of some important genes[22] and lead to various types of cancers, including ovarian can-

cer[23]. Meng et al[24] claimed that PROK1 accelerated cell proliferation and inhibited the apoptosis of rat ovary granule cells *via* PI3K/AKT/mTOR signaling way. Therefore, they pointed that PROK1 facilitated PCOS development. They also demonstrated that a microRNA; miR-28-5p bound to an untranslated region of PROK1 and impaired its expression. This inhibition prevented the occurrence of PCOS. Thus, they stated that the inhibition of PROK1 should be the touchstone of the treatment of PCOS.

There is evidence in the literature that supports the importance of vitamin D in metabolic disorders and infertility in women with PCOS. Menichini et al[25] showed the beneficial role of low dose vitamin D supplementation (400-800 IU/day) in vitamin D deficient women with metabolic problems like PCOS, in insulin resistance and endometrial receptivity which are vital for reproductive health[25].

Autoimmunity and inflammation have been closely linked to PCOS. Therefore, women with autoimmune thyroid disease struggling with infertility often present with PCOS. The similarity between zona pellucida and thyroid tissue has been shown to lead to the cross-reactivity of anti-thyroid peroxidase antibodies (TPO-Abs) toward zona pellucida[26]. Medenica et al[26] reported that cell and gene therapies for thyroid autoimmunity would be treatment options for infertility especially in women with PCOS. However, we found no statistically significant difference in PROK1 levels of the groups included in our study. The major limitation of our study is the small sample size. Therefore, there is a need for larger scale studies to clarify whether an increase in the PROK1 level leads to PCOS.

Conclusions

Although our study did not reach a conclusion to suggest PROK1 as a predictor or a predisposing factor of PCOS, it is the first study investigating the relationship between PROK1 and the pathogenesis of PCOS in humans. Nevertheless, further studies should be designed to identify the exact role of this peptide in PCOS.

Ethics Approval

The study protocol was approved by the Ethical Committee of Ankara City Hospital (E2-22-1870).

Informed Consent

All the participants provided written informed consent.

Conflict of Interest

The authors have no conflict of interest to declare.

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