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Does asporin have a role in polycystic ovary syndrome? A pilot study

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SUMMARY

OBJECTIVE: Asporin is secreted by theca cells in the mouse ovaries and is an effective marker at the gonadotropin-independent stage in secondary follicle development. It has an inhibitory effect on transforming growth factor beta and bone morphogenic proteins, which are involved in androgenesis process. Our aim was to compare serum asporin levels of polycystic ovary syndrome and control groups and examine the relationship between asporin and hyperandrogenism.

METHODS: A total of 60 patients, i.e., 30 polycystic ovary syndrome group and 30 controls, were included in the study. The demographic characteristics, hormonal status, and serum asporin levels of patients were evaluated and compared for each group. In addition, polycystic ovary syndrome patients were analyzed according to the presence of hyperandrogenism. Receiver operating characteristic curve analysis was performed for asporin levels in order to distinguish polycystic ovary syndrome patients from controls.

RESULTS: Body mass index, serum asporin and androgen levels, free androgen index, and insulin resistance values were statistically significantly higher in polycystic ovary syndrome group. Serum asporin levels were statistically significantly higher in hyperandrogenic polycystic ovary syndrome patients compared to non-hyperandrogenic polycystic ovary syndrome women (p=0.010). Receiver operating characteristic curve analysis was done for serum asporin levels to distinguish between polycystic ovary syndrome patients and healthy controls (area under the curve=0.676, standard error: 0.070, 95%CI: 0.539–0.812, p=0.019, 63.3% sensitivity, and 70% specificity).

CONCLUSION: The elevation of serum asporin levels in patients with polycystic ovary syndrome may be associated with the pathogenesis of this syndrome, or it may be the consequence of the disease. This relationship may be explained through the androgen mechanism.

KEYWORDS: Polycystic ovary syndrome. Hyperandrogenism. Asporin.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy of the reproductive age, with its incidence rate of approximately 10%[1]. PCOS not only affects the reproductive system but also causes clinical and biochemical effects by affecting many structures, such as the cardiovascular system, adipose tissue, and metabolic system[2,3]. Rotterdam criteria are the most frequently used method for diagnosis, including oligo-anovulation, hyperandrogenism, and polycystic ovary appearance in ultrasound. PCOS affects so many systems, but these effects are not included in the Rotterdam diagnostic criteria[4].

The underlying mechanism in PCOS etiopathogenesis is unclear and a real challenge[5]. Cellular studies have mostly been done on granulosa cells (GCs) and androgen metabolism disorders. Interaction between GC and oocyte plays an important role in ensuring oocyte maturation[6]. In addition to being effective in normal folliculogenesis, GCs also play a role in pathological folliculogenesis conditions such as PCOS[7]. In PCOS, dominant follicle development is impaired and GC function differs from normal in folliculogenesis steps. Increased proliferation of GCs has been demonstrated in murine PCOS models and in women diagnosed with PCOS[8,9]. During the folliculogenesis process, GC and theca cell (TC) interact and TC is where androgen production occurs in the ovary. Although the exact cause is unknown, it has been shown that TCs are overactive in PCOS, probably due to genetic/epigenetic reasons, and consequently, intraovarian androgen production is increased[10].

Asporin, which consists of 380 amino acids and belongs to the small leucine-rich repeat proteoglycan family, was identified in 2001 by three different independent groups[11-13]. It has been shown that asporin is synthesized in different tissues such as articular cartilage, periodontal ligament, connective tissues, aorta, and uterus[11-13]. Previous studies have examined the functions of asporin and concluded that asporin inhibits transforming growth factor-beta (TGF-β) in articular cartilage tissue, suppresses bone morphogenic protein (BMP)-related cytodifferentiation in periodontal ligament, affects TGF-β/SMAD2-3 pathway in colorectal cancer, and plays a role on tumor metastasis via BMP in mesenchymal stromal cells[14-17].

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Androgen synthesis in TCs takes place as a result of a complex process in which many biochemical markers play a role in steroidogenesis steps. In this process, many triggering factors such as insulin-like growth factor-1 (IGF-1)[18,19], inhibin[18], stem cell factor/kit ligand[20], as well as proteins with inhibitory effects such as TGF-β[21], BMP[22,23], and activin[24] are involved. For these reasons, any disturbance in the functioning of TCs will affect the female reproductive system.

One study in 2019 reported that asporin is secreted by TC/interstitial cells in the mouse ovaries and is an effective marker at the gonadotropin-independent stage in secondary follicle development[25]. This study found that asporin has an inhibitory effect on the TGF-β/SMAD2-3 cascade and suggested that asporin may play an autocrine/paracrine role in folliculogenesis[25]. Moreover, asporin may also affect androgen production through similar pathways.

Our hypothesis is that considering the suppressing effect of asporin on TGF-β and BMPs, it may play a role in hyperandrogenism in PCOS patients and, therefore, serum asporin levels will be high in PCOS patients. With this point of view, we aimed to compare serum asporin levels of PCOS and control groups and examine the relationship between asporin and hyperandrogenism.

METHODS

This study was conducted between July 2019 and July 2020 at Department of Obstetrics and Gynecology, Near East University. Ethics committee approval was obtained from the local ethics committee for the study (project number: YDU/2019/71-875), and informed consent was obtained from all patients.

In this pilot study, a total of 60 patients, i.e., 30 PCOS and 30 controls, were included. Post-hoc analysis of the pilot study data revealed that effect size was 1.22, and 22 individuals were required in each group for attaining 0.95 power with 0.05 alpha error probability. The diagnosis of PCOS was made according to Rotterdam criteria[4]. Medical history, age, height, weight, and blood pressure values of all patients were recorded. Body mass index (BMI) was calculated by dividing body weight in kilograms by the square of height in meters. All patients were nulligravid, and transvaginal ultrasonography was performed on all of them after gynecological examination within the first 5 days of menstruation. In patients with oligomenorrhea after pregnancy was ruled out, menstruation was induced by administering 5-mg medroxyprogesterone acetate twice a day to create progesterone-withdrawal bleeding.

Serum glucose, insulin, thyroid-stimulating hormone (TSH), prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), androstenedione, dehydroepiandrosterone sulfate (DHEAS), sex hormone-binding globulin (SHBG), free testosterone (fT), and total testosterone (TT) values were analyzed from the blood sample taken in the morning fast from all patients. One tube of these blood samples was centrifuged and stored at −80°C for each participant until the day when serum asporin level is measured.

The free androgen index (FAI) was obtained by multiplying the ratio of serum total testosterone level to serum SHBG level by 100 (i.e., 100×TT/SHBG). Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) was calculated as follows: fasting glucose × fasting insulin/405.

Serum asporin level was analyzed with Human Asporin sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) assay (catalog no.: abx150758, Abbexa Ltd., Cambridge, UK).

Exclusion criteria were as follows: age >35 and <18 years, hyperprolactinemia, thyroid dysfunction, pregnancy status, congenital adrenal hyperplasia, use of drugs that affect the hypothalamic-ovarian axis or hormones, history of ovarian surgery, use of any hormones including combined oral contraceptives in the past 6 months, having a disease affecting the skeleton and cartilage system, any malignancy, smoking, and alcohol use. The presence of follicles larger than 10 mm in the ovary or the detection of ovarian cysts was also considered exclusion criteria.

Statistical analysis

Social Sciences Statistics Program (SPSS) version 16 was used for statistical analysis. Kolmogorov-Smirnov test was performed to show the distribution of data. Mann-Whitney U test was used for continuous variables that have non-normal distributed. Data were expressed as median (interquartile range) and p-values. For correlation of asporin and androgens, Spearmen's correlation was performed. A $p<0.05$ was considered statistically significant.

RESULTS

The demographic characteristics and laboratory results of the patients with PCOS and the healthy group are shown in Table 1. BMI, asporin, LH/FSH, DHEAS, androstenedione, free testosterone, FAI, and HOMA-IR values were statistically significantly higher in PCOS group.

When PCOS patients were divided into two groups according to their hyperandrogenism status, we found that serum asporin levels were significantly higher in the hyperandrogenic PCOS group (Table 2).

There was no statistically significant correlation between the serum androgen levels and asporin in PCOS patients with hyperandrogenism. In addition, correlation analysis was performed between BMI and asporin for both PCOS and healthy patients, but no correlation was detected.

Table 1. Comparison of polycystic ovary syndrome and healthy groups.

	PCOS (n=30)	Control (n=30)	p
Age	22.00 (21.0–25.0)	23.50 (21.0–25.25)	0.633
BMI (kg/m^2)	22.7 (20.45–25.55)	20.19 (19.21–21.93)	0.003
Asporin (ng/mL)	31.85 (7.46–51.45)	19.31 (2.91–29.88)	0.019
LH/FSH ratio	1.34 (0.87–2.13)	0.91 (0.83–1.10)	0.004
DHEAS (μg/dL)	304.55 (229.38–400.40)	167.65 (142.80–275.98)	<0.001
Androstenedione (ng/dL)	136.70 (91.55–175.22)	70.40 (61.83–78.70)	<0.001
Free testosterone (pg/mL)	1.56 (1.16–2.32)	0.91 (0.80–1.05)	<0.001
FAI	3.01 (1.85–4.93)	1.10 (0.84–1.37)	<0.001
HOMA-IR	1.70 (1.10–2.60)	1.27 (0.89–1.45)	0.001

BMI: body mass index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, DHEAS: dehydroepiandrosterone sulfate, FAI: free androgen index, HOMA-IR: Homeostatic Model Assessment-Insulin Resistance. Bold indicates statistically significant values.

Table 2. Comparison of polycystic ovary syndrome patients according to hyperandrogenism.

	HA+ (n=19)	HA– (n=11)	p
Age	22.00 (21.0–24.0)	24.00 (22.0–27.0)	0.127
BMI (kg/m^2)	22.5 (20.8–25.5)	23.40 (20.3–26.7)	0.846
Asporin (ng/mL)	43.12 (24.88–58.02)	6.85 (0.74-42.78)	0.010
LH/FSH ratio	1.38 (0.87–2.12)	1.27 (0.86–2.17)	0.846
HOMA-IR	1.70 (1.0–2.09)	1.10 (1.81–2.90)	0.576

BMI: body mass index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, HOMA-IR: Homeostatic Model Assessment-Insulin Resistance. Bold indicates statistically significant values.

Receiver operating characteristic (ROC) curve analysis was made for serum asporin levels in distinguishing between PCOS patients and healthy controls (area under the curve=0.676, standard error: 0.070, 95%CI 0.539–0.812, p=0.019; 63.3% sensitivity and 70% specificity). The comparison of the ROC curve of serum asporin is shown in Figure 1.

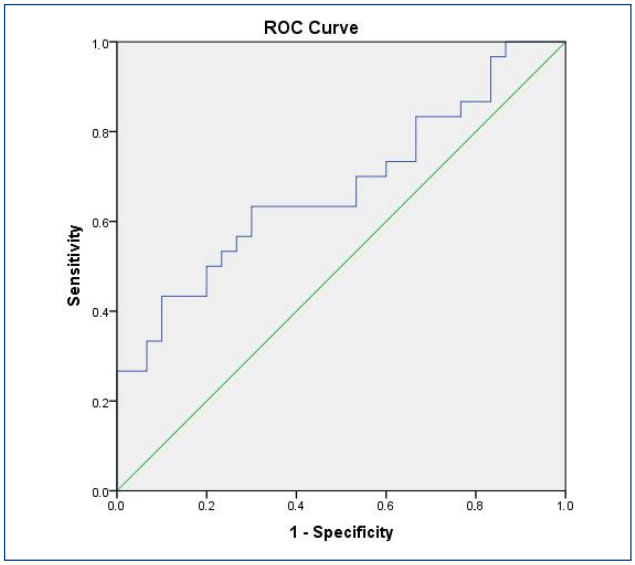

Figure 1. Receiver operating characteristic curves of serum asporin for differentiating polycystic ovary syndrome patients from healthy women.

DISCUSSION

It has long been known that hyperandrogenism, obesity, and high insulin resistance are more common in PCOS. In our study, significant increases were found in the PCOS group in terms of these data as expected. However, the most important result of this study is that the serum level of asporin, which is synthesized in TCs and stated to play a role in folliculogenesis steps, was significantly higher in patients with PCOS compared to healthy women.

Studies on asporin in the past mostly focused on cartilage tissue, bone tissue, and cancer. Unlike the examples in the literature, Aoyama et al. investigated that asporin is secreted from TCs in the mouse ovarian and stated that it may have a role in folliculogenesis[25]. In this study, it has been shown that asporin antibody arrested secondary follicle development and induced the TGF-β signaling[25]. With these findings, it was concluded that asporin has an inhibitory effect on TGF-β and plays an

important role in secondary follicle development[25]. TGF-β has been shown to suppress androgen production in TCs of humans[21]. Also, TGF-β plays a role in steroidogenesis steps in TCs and dysregulation of this molecule is one of the responsible factors for increase in stromal thickness and hyperandrogenism in PCOS[26]. Asporin, which was found to be high in PCOS patients in our study, may play a role in the pathogenesis of this syndrome by contributing TGF-β pathway.

Theca cells have been relatively neglected in PCOS pathogenesis studies. There are many molecules involved in the stages of steroidogenesis and folliculogenesis in TCs. For example, BMPs have an inhibitory effect on the steps from cholesterol to androstenedione production in TCs[27]. Asporin, which affect the cytodifferentiation in different tissues via BMPs, can have a similar effect in ovaries[14-17]. Tomoeda et al. have concluded that asporin binds BMP[15]. Glister and Campbell reported that BMPs 2/4/6/7 all significantly decreased androstenedione production from TCs[22,23]. In the light of these data, the findings of Aoyama et al.[25] and results of our study can conclude that asporin may have a role in androgen production in TCs by suppressing BMPs. In our study, although serum asporin levels were found to be significantly higher in hyperandrogenic PCOS patients compared to normoandrogenic PCOS patients, the lack of correlation between serum asporin and androgen levels may be due to the relatively low number of patients.

There are some studies in the literature that reveal the relationship between asporin and cartilage tissue. It has been stated in the literature that asporin is expressed in cartilage tissue and is associated with osteoarthritis, and this relationship is mediated by TGF-β[28-31]. In addition, studies have shown that cartilage tissue is thicker in PCOS patients than in healthy women; nevertheless, the possibility of osteoarthritis is increased in relation to hyperandrogenism[32,33]. Considering the fact that asporin and hyperandrogenism seen in PCOS are associated with cartilage tissue, the high serum asporin levels that we detected in the PCOS group in our study may not be solely of ovarian origin, and further studies are needed to reveal this issue.

Our study was the first to show that serum asporin level was significantly higher in PCOS patients, although its mechanism is not known exactly. PCOS is a syndrome with a complex pathogenesis and many unknowns. The finding of this study may be a step in revealing the pathogenesis of PCOS. This study consisted of well-diagnosed patient and control groups. However, the small number of participants and the fact that other markers such as TGF-β that may be affected by asporin have not been studied can be considered the limitations of this study.

CONCLUSIONS

According to results of our study, the elevation of serum asporin levels in PCOS patients may be associated with the pathogenesis of this syndrome. This relationship might be due to the androgen mechanism. Further studies with high patient numbers are needed to elucidate this situation, especially involving the patients with hyperandrogenic PCOS phenotypes.

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AUTHORS' CONTRIBUTIONS

YÖ: Conceptualization, Formal Analysis, Funding acquisition, Methodology, Validation, Visualization, Writing – review & editing. **ACÖ:** Data curation, Formal Analysis, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **ÖEÖ:** Data curation, Investigation, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **OE:** Data curation, Formal Analysis, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing.

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