Reproductive Medicine; *Endocrinology and Infertility*

Does the Serum Vaspin Level Have a Role in the Diagnosis of Primary Ovarian Insufficiency?

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ABSSTRACT

OBJECTIVE: This study was designed to compare the serum vaspin levels between patients with primary ovarian insufficiency (POI) and healthy fertile women.

STUDY DESIGN: Sixty-nine patients diagnosed with idiopathic POI and 70 age-matched healthy fertile women were included in this case-control study. General gynecological and physical examination findings and serum basal hormone levels were recorded. Vaspin levels in serum samples were determined using the Enyzme-Linked ImmunoSorbent Assay (ELISA) method.

RESULTS: The mean age of the patients with POI was 31.9±5.4 years, while it was 31.2±3.8 years in the control group. There were no statistically significant differences in body mass index, gravidity, parity, and cigarette consumption between the groups. However, the mean follicle-stimulating hormone level in POI patients was 55.4±25.1 IU/L, significantly higher than the 6.2±1.8 IU/L observed in the control group. Luteinizing hormone levels were also significantly higher in POI patients, while estradiol and anti-Mullerian hormone values were significantly lower. Furthermore, the mean serum vaspin level in POI patients was 0.79±0.56 ng/mL, compared to 1.08±0.64 ng/mL in the control group (p<0.001).

CONCLUSIONS: Serum vaspin levels were found to be decreased in women with POI. Therefore, monitoring the serum vaspin levels in women with POI may help in the early identification of patients with POI.

Keywords: Infertility; Ovarian aging; Primary ovarian insufficiency, Vaspin

Introduction

Primary ovarian insufficiency (POI) has no definitive diagnostic criteria. POI is defined as the detection of two follicle-stimulating hormone (FSH) values greater than 25 IU/L in women with oligo/amenorrhea for more than four months under the age of 40 as to the European Society of Human Reproduction and Embryology (ESHRE). The incidence of POI is not as low as it is thought. It affects 1% of women under 40 years of age (1).

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Some potential factors of POI etiology are genetic anomalies, autoimmune causes, metabolic disease, iatrogenic causes, infections, and environmental factors. The mechanisms put forward in the etiopathogenesis of POI are the lack or absence of ovarian reserve and the increase in the rate of ovarian follicular atresia. Moreover, follicular atresia is a complex condition with multiple regulators and can be activated by various pathways, leading to the termination of ovarian follicles. However, the etiology of POI still has not been elucidated (2,3).

Visceral adipose tissue-derived serine protease inhibitor (vaspin), was shown to be expressed in the liver, pancreas, cerebrospinal fluid, hypothalamus, intestines, lungs, and ovaries, its origin in adipose tissue (4,5). Vaspin has also been shown to affect ovarian steroid synthesis and ovarian proliferation, similar to other adipokines, such as leptin, resistin, and apelin (5). It has been shown to significantly increase granulosa cell proliferation and progression of the cell cycle, as well as reduce apoptosis (6). Additionally, vaspin was shown to be a novel modulator of granulosa cell physiology in humans. However, the role of vaspin in the human reproductive system is still unclear (7). Apoptosis has a place in the pathogenesis of POI (8). Our aim in this study was to contribute to the literature in terms of guiding the diagnosis and treatment steps by determining the relationship of vaspin, which is known to be anti-apoptotic, in POI patients.

Material and Method

Approval was obtained from the institutional ethics committee (27.02.2020/E1/349). Between February and July 2020, patients diagnosed to be POI as to ESHRE diagnostic criteria who applied to the Reproductive Endocrinology Department of a tertiary center were enrolled in this case-control study (1). Written informed consent was obtained from all participants.

Patients with a family history of POI, karyotype anomalies, and genetic diseases were excluded from the study. Patients who have general systemic diseases (such as liver, pancreas, and lung), who have undergone ovarian surgery, chemotherapy, and radiotherapy were excluded. In addition, patients on hormone replacement therapy or who had previously undergone infertility treatment, endocrine pathology, autoimmune diseases, endometriosis, and polycystic ovary syndrome were excluded. Body mass index (BMI) >30 kg/m^2 and <18 kg/m^2 were among the study exclusion criteria.

Women who were admitted to the Family Planning or Gynecology Clinic for contraception or routine gynecological examination were included as the control group. The control group was randomly selected from fertile patients aged 18-40 years, with regular menstrual cycles, no comorbidities, and no history of drug use in the last three months.

General physical and pelvic examinations were conducted for each patient. Demographic characteristics, age, BMI, cigarette consumption, and obstetric, and gynecological background of each patient were recorded.

Regarding the study parameters, FSH, estradiol (E2), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), prolactin levels, and serum vaspin levels were recorded. Anti-mullerian hormone (AMH) levels were also recorded.

Venous blood samples of the participants who still had menstrual cycles were taken on the 2nd or 3rd day of the menstrual cycle. Blood samples were taken on a random day from amenorrheic patients. The samples reached the laboratory within 10 minutes. For the measurement of serum hormone levels, the UniCel DxI 800 Immunoassay System was used. After centrifuging the blood samples, the serum was separated and stored at -80°C until the day of analysis. The enzyme-linked immunosorbent assay (ELISA) method was used to measure vaspin levels using a commercially prepared kit (Human Elisa Kit/96*2 test, Cloud Clone Wuhan USCN Business Co., Ltd.). Ng/mL was used to express the results of the vaspin levels. The intra- and inter-assay coefficients of variation of the kit were lower than 10%.

Statistical analysis

Statistical Package for the Social Sciences 22 program (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Kolmogorov-Smirnov test and Shapiro-Wilk test were used for the conformity of the data to the normal distribution. Whereas nominal data with normal distribution was shown as mean ± standard deviation, non-normally distributed nominal data was shown as median [minimum-maximum]. Numbers (%) were used to show the categorical data. The Independent-Sample t-test was used in the analysis of variables with normal distribution, and the Mann-Whitney U test was used in the analysis of non-normally distributed variables. The comparison of categorical data was tested with Pearson Chi-Square. The presence of correlation between parametric data was tested using Pearson correlation coefficient and the correlation between non-parametric or without normal distribution data was tested using Spearman correlation coefficient. Data were examined at a 95% confidence level. A p-value less than 0.05 was accepted as significant.

Results

One hundred thirty-nine patients, 69 of whom were diagnosed with POI and 70 healthy controls, were enrolled in this study.

The distribution of some descriptive, clinical, and laboratory findings of the patient groups is shown in Table I. The mean age of the patients with POI was 31.9±5.4 years, it was 31.2±3.8 years in the control group (p=0.201). BMI, gravidity, parity, and cigarette consumption in patients with POI and control group patients were similar (All p>0.05). Whereas the mean FSH level in POI patients was 55.4±25.1 IU/L, it was 6.2±1.8 IU/L in the control group. In addition, while the LH levels of the patients with POI were higher than the controls, E2 and AMH values were lower (p<0.05) (Table I). All the patients were married and none of them used alcohol during their lifespan.

Primary ovarian insufficiency patients' mean serum vaspin level was 0.79±0.56 ng/mL, while it was 1.08±0.64 ng/mL in the control group (p<0.001). A statistically significant difference was found as to serum levels of vaspin between the groups (Table I).

Correlations between vaspin and the evaluated parameters were determined. There was no correlation between the vaspin levels and age, basal hormone levels, body mass, BMI, number of gravida, and parity in POI patients (All p>0.05).

The mean serum vaspin levels of smoker POI patients (n=14) were significantly lower than the control patients who smoked (n=12) (0.86±0.83 ng/mL vs 1.32±1.00 ng/mL, respectively) (p=0.017). No significant statistical difference in serum vaspin levels was found when smoker POI patients were compared to non-smoker POI patients (0.86±0.83 ng/mL vs. 0.92±0.84 ng/mL, respectively).

Table I: *Descriptive, clinical features, laboratory findings, and serum vaspin levels of the patients*

Variable	POI (n=69)	Controls (n=70)	p
Age (year)	31.9±5.4	31.2±3.8	0.201
Body Mass (kg)	62 (49-81)	61 (48-71)	0.276
BMI (kg/m²)	23.6±2.4	23.1±2.2	0.205
Gravida (number)	1 (0-5)	1 (0-4)	0.280
Parity (number)	0 (0-3)	1 (0-3)	0.073
Abortus (number)	0 (0-2)	0 (0-3)	0.900
FSH (IU/L)	55.4±25.1	6.2±1.8	<0.001
LH (IU/L)	27.1±14.4	6.1±3.8	<0.001
Estradiol (pg/dL)	19 (11-41)	100 (31-280)	<0.001
AMH (ng/ml)	0.08±0.16	3.57±2.01	<0.001
TSH (mIU/L)	2.25±1.16	2.28±1.82	0.834
Cigarette smokers	14 (20.3%)	12 (17.1%)	0.634
Vaspin (ng/mL)	0.79±0.56	1.08±0.64	<0.001

Values were given as mean±standard deviation, median [min-max], and number (%).
POI: Primary ovarian insufficiency, BMI: Body Mass Index, FSH: Follicle Stimulating Hormone, LH: Luteinizing Hormone, AMH: Anti-mullerian hormone, TSH: Thyroid Stimulating Hormone, p<0.05 was considered statistically significant.

Discussion

Primary ovarian insufficiency is a heterogeneous disease affecting 1% of women in the reproductive period (1). Since POI increases morbidity in women and increases workforce loss and also healthcare costs, early diagnosis, and treatment are of great importance. Vaspin, which is derived from adipose tissue, has been shown to prevent apoptosis. Kurowska et al. have shown cell cycle-dependent vaspin expression in ovarian follicles and other tissues (5). In their subsequent study, they showed that vaspin was also effective in the anti-apoptotic and proliferative effects in porcine granulosa cells(6).

We thought that alterations in serum vaspin levels may be related to POI due to its role in the follicular apoptosis mechanism. Therefore, in this study, we aimed to investigate whether serum vaspin levels could serve as a parameter to support POI diagnosis. In this study, we found that serum vaspin levels in POI patients were significantly lower than those in the control group (p<0.001). Additionally, we also observed that vaspin serum levels were remarkably lower in smoker POI patients than the smoker control patients.

Measuring the level of serum vaspin enzyme is one of the methods we think, is suitable for practical clinical use. There is no study in the literature evaluating serum vaspin levels in POI patients. However, there are studies examining the effect of vaspin and other serum biomarkers that may affect the female reproductive system, especially idiopathic POI (9,10). In a study held by Dogan et al., a positive correlation between serum vaspin levels and PCOS regardless of BMI was shown (11). In a systemic review of 88 studies, the results showed that serum levels of vaspin were higher in the PCOS group than in controls (12). It has been reported that the formation of multicystic ovarian tissue, as well as hyperplasia in the theca and granulosa cells, were among the mechanisms involved in

the pathophysiology of PCOS (13). Vaspin shows an anti-apoptotic effect by stimulating granulosa cell proliferation in folliculogenesis on theca and granulosa cells (6). Therefore, it is thought that increased vaspin levels in PCOS may play a role in theca and granulosa cell hyperplasia. In our study, vaspin levels were significantly lower in the POI group. These results obtained from our study suggest that the decrease in cell proliferation and increased apoptotic effect due to decreased vaspin may cause depletion in the follicles by disrupting the folliculogenesis process. Thus, may have a role in the etiology of POI.

Vaspin has also been demonstrated to have protective effects on the cardiovascular system. It has been shown to prevent the occurrence of vascular complications and protect vascular endothelial cells from fatty acid-induced apoptosis (14). In vitro animal studies have shown that vaspin attenuates TNF-α-induced apoptosis and improves cardiac dysfunction in rats with diabetic cardiomyopathy (15). Furthermore, another study reported that serum vaspin levels were an important predictor for the diagnosis of patients with cardiovascular system disease. In addition, low serum vaspin levels were found to be an independent predictive value for acute myocardial infarction and heart failure in this study group (16). It has been shown that the risk of cardiovascular disease was increased in POI patients (17). Therefore, we can think that the decreased vaspin levels we found may also be associated with the increased risk of cardiovascular disease in POI patients.

It was shown that serum vaspin level increases as BMI increases (18). However, no correlation between the vaspin level and BMI in POI patients was found in our study. This result may be due to the similar anthropometric characteristics of the patients in our study.

Tobacco toxins reduce fertility by affecting folliculogene-

sis, oogenesis, embryo transport, implantation, uterine flow, and myometrial growth. Tobacco toxins both inhibit the synthesis of estrogen and impair the endocrine effect, thereby reducing the age of menopause in women (19). In our study, serum vaspin levels of smokers and non-smokers in the POI group were similar. In the current study, the patients were asked if they currently smoked cigarettes. And they were classified as smokers and non-smokers. However, we do not know whether they used to smoke but have since stopped smoking. Therefore, the insignificant difference between the smoker and non-smoker POI patients' vaspin concentrations can be due to this lack of evidence. Also, the low number of smoker patients may have led to a limitation.

The strength of this current prospective study is that we found no significant difference in age and BMI between the groups. So, the factors that can affect the serum vaspin levels were eliminated. Additionally, this study differs from a fertile control group. This is the first study to show that serum vaspin was lower in POI patients. Therefore, we think, it will contribute to the literature.

However, there are several limitations. One limitation of the study is the relatively small sample size. Moreover, considering that vaspin levels could potentially be reduced in somatic cells such as blood samples, it would have been beneficial to investigate serum vaspin levels concurrently with ovarian tissue vaspin levels. Furthermore, a third group could have been formed by correcting the age factor and including menopausal patients in this study. Since we thought that vaspin levels impair steroidogenesis, observing the lowest levels in menopausal patients would make the study more valuable.

Conclusion

In conclusion, in the current study, the serum level of vaspin was found to be statistically significantly lower in POI patients. The decrease in vaspin level may be one of the factors in the etiopathogenesis of POI. In this way, regarding the long-term effects and results of serum vaspin levels and POI on women's health, future patient morbidities can be reduced by aiding early diagnosis. However, further studies are needed to apply the validity of these findings in the diagnosis of POI in the clinics.

Ethical Approval and Informed Consent: All procedures performed were in accordance with the ethical standards of the institutional committee (27.02.2020/ E1/349), and with the 1964 Helsinki Declaration. All participants gave written informed consent after they were informed about the study.
Availability of data and materials: The data supporting this study is available through the corresponding author upon reasonable request.
Disclosure statement: The authors declare that they have no conflict of interest.
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