Original Article

There is No Association between Premature Ovarian Insufficiency and Levels of Fetuin-A/α2-Heremans-Schmid Glycoprotein

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Abstract

Objective: Fetuin-A is a well-known negative acute-phase protein and has been used liberally to predict vascular disease. The aim of this study was to evaluate the association between serum human fetuin-A/alpha2-Heremans–Schmid glycoprotein levels and idiopathic premature ovarian insufficiency (POI). **Methods:** A total of 75 women were included in this case–control study between January 2013 and December 2013. Serum fetuin-A concentrations were measured in 36 women with idiopathic POI and 39 healthy women with regular cycles. Blood samples were drawn after a 12-h overnight fast and were kept at −80°C for subsequent assay. The serum levels of fetuin-A were assessed by commercial ELISA kits (BioVendor Laboratory Medicine Inc., Brno, Czech Republic) and serum concentration values were expressed as μg/ml. **Results:** The mean serum fetuin-A levels of idiopathic POI and control women were 229.02 ± 27.79 and 232.37 ± 65.56, respectively, with $P = 0.771$ (independent samples *t*-test). Our results showed no statistically significant difference between serum fetuin-A levels of idiopathic POI women and controls. **Conclusion:** The mean values of serum fetuin-A in idiopathic POI women were not significantly different from controls, which implies that there is no significant association between serum fetuin-A levels and idiopathic POI.

Keywords: *Fetuin-A, inflammation, premature ovarian insufficiency*

Introduction

Premature ovarian insufficiency (POI) is hypergonadotropic hypoestrogenic amenorrhea seen in 0.3%–1.2% of women before the age of 40 years.[1-4] There is not a clear consensus on the definition of POI and follicle-stimulating hormone (FSH) levels; according to the European Society of Human Reproduction and Embryology, POI is defined as the loss of ovarian activity before the age of 40 years, characterized with menstrual disturbance (amenorrhea and oligomenorrhea), raised gonadotropins and low estradiol.[1] Guideline development group recommends the diagnostic criteria as: women under 40 years of age experiencing symptoms of amenorrhea/oligomenorrhea for 4–6 months and FSH levels >25 mIU/ml measured >4 weeks apart are confirmed for the diagnosis of POI.[1] There are three presumable mechanisms involved in the development of POI; apoptosis acceleration, follicular maturation blocking, and premature follicle activation.[5] POI has a multifactorial etiology: familial history, genetic abnormalities, autoimmune conditions, iatrogenic damage of ovaries, and idiopathic causes.[6-9] Recent studies showed that some inflammatory markers may serve as a diagnostic marker in POI.[10]

Fetuin-A, which is also known as α-2-Heremans–Schmid protein, is a well-known negative acute-phase protein (APP), mainly produced by the liver and to a lesser extent by the placenta and tongue.[11,12] Fetuin-A takes part in insulin signaling pathways, vascular

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calcification, metabolic disorders, central nervous system, cardiovascular system, and pro-inflammatory pathways.[13,14] Fetuin-A was reported to take part in molecular pathways of oocyte maturation and existed in human follicular fluid.[15]

The aim of this study was to evaluate the association between human fetuin-A/α-2-HS glycoprotein levels and idiopathic POI considering the mechanism that is possibly related with inflammation.

Methods

Seventy-five women who were referred to our reproductive endocrinology outpatient clinic between January 2013 and December 2013 were enrolled in this prospective cohort study. The institutional review board (9/23/2013 #15) approved the study and written informed consent for participation in the study was obtained from all the participants. Serum fetuin-A concentrations were measured in 36 women diagnosed with idiopathic POI and 39 healthy women with regular menstrual cycles. The idiopathic POI group selection criteria were determined as women between the ages of 20–40 years, experiencing amenorrhea within the past 4 months, and with FSH levels higher than 40 mIU/Ml recorded two times during measurements within at least 1 month apart. Patients having hormone therapy, history of pelvic surgery, exposure to radiotherapy and chemotherapy, abnormal karyotype analysis in participants younger than 30 years of age, and any systemic disease such as thyroid disorders or hyperprolactinemia were excluded from the study. Women aged 20–40 years with regular menstrual cycles who were not using hormonal contraception and without a history systemic disease or drug use were included in the control group. Blood samples were drawn after 12-h overnight fasting and were kept at −80°C for subsequent assay. The serum levels of fetuin-A were assessed by commercial ELISA kits (BioVendor Laboratory Medicine Inc., Brno, Czech Republic) and serum concentration values were expressed as μg/ml. Complete blood count, fasting plasma glucose (FPG), FSH, luteinizing hormone, estradiol, prolactin, and thyroid-stimulating hormone (TSH) levels were evaluated in all participants.

Demographic variables and fetuin-A results were presented as mean with standard deviation or median with range for continuous variables. The univariate analyses were investigated using the Student's (independent samples) *t*-test or the Mann–Whitney U-test to compare continuous variables as appropriate. Pearson correlation coefficients were calculated for continuous variables with normal distribution, and Spearman rank correlation coefficients were calculated for non-normally distributed continuous variables. A two-tailed $P < 0.05$ was considered statistically significant. The statistical software package SPSS 23.0 (SPSS Inc., Chicago, Ill., USA) was used for the data analyses.

Results

Patients were statistically similar in terms of age, body mass index, FPG, TSH, prolactin and hemoglobin levels, leukocyte (white blood cell [WBC]) count, and neutrophil-to-lymphocyte ratio (NLR) ($P > 0.05$) [Table 1].

The mean serum fetuin-A levels of POI and control women were 229.02 ± 27.79 μg/mL and 232.37 ± 65.56 μg/mL, respectively, with $P = 0.771$ (independent samples *t*-test) [Figure 1]. There was no correlation between fetuin-A and age, body mass index, FPG, TSH, prolactin and hemoglobin levels, leukocyte (WBC) count, and NLR ($P > 0.05$). Our results showed no statistically significant difference and correlation between serum fetuin-A levels of POI women and controls.

Discussion

This is the first study investigating the association between POI and fetuin-A. Fetuin is defined as a multifunctional protein which was first isolated from bovine in 1944.[16] Intensive researches on this liver-synthesized protein unveiled that fetuin-A had various functions on metabolism, central nervous system, cardiovascular system, and bone and mineral metabolism.[17] Fetuin-A acts as a negative APP in infection and as a positive APP in injury.[11]

The inflammation-associated pathogenesis of POI is investigated by several studies. Since the mechanism that induces ovarian autoimmunity and inflammation is still

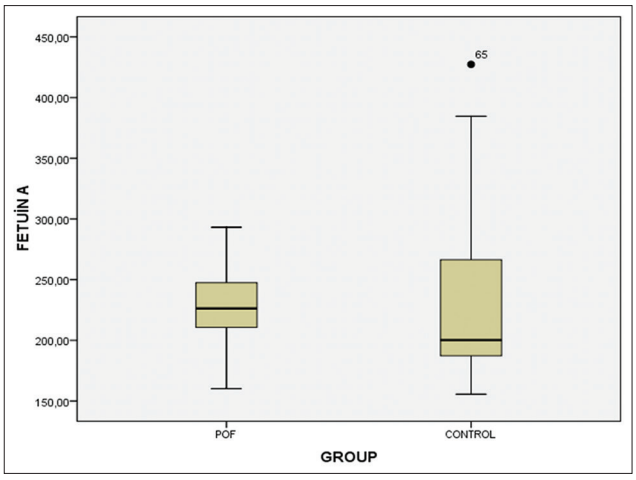

Figure 1: Serum fetuin-A levels in premature ovarian insufficiency and control group

Table 1: Demographic variables and serum fetuin-A levels of premature ovarian insufficiency and control group

Mean±SD (minimum-maximum)	Control	POI	*P**
Age (years)	31.9±5.6 (21-42)	34.1±4.9 (22-39)	0.077
BMI (kg/m^2)	26.4±4.7 (19.1-39.1)	24.8±3.9 (18.4-34.7)	0.149
Hemoglobin (g/dL)	13.1±1.3 (10.2-15.4)	13.7±1.2 (9.2-15.7)	0.073
Prolactin (ng/mL)	13.5±7.7 (4.9-31)	10.1±5.0 (4.9-29.1)	0.131
FSH (IU/mL)	7.5±1.5 (5.4-9.8)	60.6±26.4 (40-149)	<0.001
E2 (pg/mL)	92.8±57.2 (42.7-229.7)	29.2±21.9 (11-90)	0.001
TSH (U/mL)	1.7±1.1 (0.2-3.6)	1.9±1.0 (0.3-4.3)	0.698
FPG (mg/dL)	90.27±8.4 (72-104.3)	90.33±11.6 (75-121)	0.985
WBC (10^9/L)	7.2±1.2 (5.1-10.5)	6.6±1.8 (3.7-11.9)	0.144
Serum fetuin-A (µg/mL)	232.4±65.6 (155.5-427.4)	229.0±27.8 (160.2-293.2)	0.771

*P<0.05 considered significant. POI=Premature ovarian insufficiency, BMI=Body mass index, WBC=White blood cell, FSH=Follicle-stimulating hormone, TSH=Thyroid-stimulating hormone, SD=Standard deviation, FPG=Fasting plasma glucose

unknown, some of the findings by these studies supported this inflammation hypothesis. Lymphoplasmacellular infiltration around steroid-producing cells in POI patients in association with adrenal autoimmunity may be an evidence for autoimmune oophoritis which is set forth to common autoantigens.[18] Ovarian tissue damage due to the viral infection may be another cause of inflammatory pathogenesis.

Yildirim *et al*. studied inflammatory biomarkers in POI patients and found a decreased NLR, whereas C-reactive protein and serum amyloid-A protein had no significant difference between the women with normal menstrual cycles and POI patients.[10]

In the literature, a respectable amount of studies have been reported on a possible relationship of fetuin-A with obesity, polycystic ovary syndrome, metabolic syndrome, fatty liver disease, diabetes mellitus, and atherosclerosis.[19-21] When metabolic profile was studied in patients with POI, POI was found to have increased risk of metabolic syndrome independent of age and obesity.[22] In terms of fetuin-A, although a relationship with pathophysiology of metabolic disorders existed in literature, the results were heterogeneous about its significance. Moreover, our results showed no relation with increased risk of metabolic syndrome in patients with premature menopause.

It was shown that fetuin-A was present in human follicular fluid and had inhibitory effects on oocyte maturation[23] and had a role in mitogenic pathways through insulin receptors in hamster ovary cells.[15] When serum and follicular fluid fetuin-A levels were analyzed in patients undergoing *in vitro* fertilization (IVF) treatment, both the levels were found higher in the IVF group[24] which also suggested the relationship of fetuin-A with infertility.

Mathur *et al*. studied fetuin-A in patients with endometriosis and concluded that the increased fetuin-A levels in serum and peritoneal fluid samples of the patients with endometriosis may have a role in autoimmune pathophysiology of the disease, which lead to decreased ovarian reserve and infertility.[25-27]

Høyer *et al*. showed fetuin mRNA and protein in granulosa cells; however, the pattern of staining differed between growing healthy follicles and atretic follicles. Depending on the macrophage-like behavior of granulosa cells in follicular atresia, they hypothesized that fetuin may have a role in the regulation of follicular growth, differentiation, and atresia.[28-31] Recent studies have been made, but a clear evidence has not been reported on a possible relation between fetuin-A and infertility. Bódis *et al*. compared serum and follicular fluid fetuin-A levels of patients receiving IVF treatment and healthy controls and found that fetuin-A was markedly elevated in serums of IVF patients; however, they reported that fetuin-A could not be used as a direct marker for the estimation of fertilization success.[24] Fetuin-A was assessed in blood samples of patients undergoing IVF treatment in a study by Yen *et al*. and was found significantly higher in the pregnant group, which would propose a possible predictive value for achieving live birth in IVF treatment in future.[32]

Taking into consideration of this studies that reported the role of fetuin-A in inflammation and autoimmunity in endometriosis and the presence of fetuin in granulosa cells in follicles which may have a role in atresia, we hypothesized that the levels of plasma fetuin-A might have a difference between the women with normal menstrual cycles and patients with POI. However, our results did not have a significant difference between the study and control groups. Further studies are needed to define the pathogenesis of early follicular atresia and the role of fetuin-A in follicular physiology which is not yet explained by the existing literature.

Conclusion

The mean values of serum fetuin-A in idiopathic POI women were not significantly different from controls, which implies that there is no significant association between serum fetuin-A levels and idiopathic POI.

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Conflicts of interest

There are no conflicts of interest.

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