

Serum pregnancy-associated placental protein-a (PAPP-A) levels are increased in polycystic ovary syndrome (PCOS) in women with oligo-anovulation

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Abstract

Background/Aim: Pregnancy associated placental protein-A (PAPP-A) is a zinc-binding metalloproteinase with a key role in insulin like growth factor (IGF) pathway, and potential atherogenic effects. There is little information in the literature regarding PAPP-A levels in Polycystic ovary syndrome (PCOS). We aimed to investigate the serum PAPP-A levels among non-obese women with PCOS as a cardiovascular risk marker.

Methods: Non-obese women of reproductive age (18-35 years of age) diagnosed with PCOS according to Rotterdam Consensus Conference criteria were included in this case-control study. Serum PAPP-A levels were compared with independent samples t-test between two main groups (PCOS and control) and investigated in PCOS subgroups as PCOS patients were further classified according to main phenotypes (hyperandrogenism and oligo-anovulation).

Results: A total of 41 women with PCOS and 40 age- and body mass index- matched controls were included in the analysis. The serum PAPP-A levels of the control and PCOS groups, and of PCOS patients with hyperandrogenism were similar ($P=0.128$, $P=0.261$, respectively). However, the serum PAPP-A levels of those with oligo-anovulation was higher than those without ($P=0.006$), and that of women without oligo-anovulation was comparable to that of the control group ($P=0.613$).

Conclusion: Lean and young PCOS women with oligo-anovulation had increased serum levels of PAPP-A when compared to women without. Prospective studies are needed to uncover the long-term cardiovascular risk of elevated PAPP-A levels in PCOS women with oligo-anovulation.

Keywords: Polycystic ovary syndrome, Cardiovascular disease, Pregnancy associated placental protein-A, PAPP-A

Introduction

The most prevalent reproductive-age endocrine disease is polycystic ovary syndrome (PCOS) [1]. It is a multisystemic and heterogeneous disease with an estimated prevalence of 5-15% that depends on the applied criteria [2]. PCOS is diagnosed when oligo-anovulation, polycystic ovarian morphology (PCOM), biochemical and/or biochemical hyperandrogenism (HA) and their combinations are present, which forms the phenotypes [3]. Besides its reproductive complications such as oligo-anovulation and infertility, PCOS also carries a considerable risk of future medical comorbidities and long-term risks including insulin resistance, diabetes mellitus, hypercholesterolemia, cardiovascular disorders, and microvascular dysfunction [4-6]. To date, the exact pathophysiology of the development of PCOS and PCOS phenotypes is poorly understood. Therefore, the interplay between the PCOS phenotypes and long-term comorbidities remain to be elucidated.

Cardiovascular diseases (CVD) continue to be the major causes of female mortality [7]. Although they usually occur mostly in late reproductive and postmenopausal periods, risk factors begin to affect patients from a young age. Evaluation of conventional cardiovascular risk factors, such as lipid profiles, blood pressure, glucose levels, insulin resistance and anthropometric measures such as body mass index (BMI) in these cases is critical in the follow-up and in later years [8]. However, there is scarce evidence for the association of novel markers indicating future CVD risk with PCOS phenotypes.

Pregnancy associated placental protein-A (PAPP-A) is a zinc-binding metalloproteinase that belongs to the Metazincin family, which interacts with the pathway of insulin like growth factor (IGF). PAPP-A was first isolated from plasma during human pregnancy in 1974 [9]. Further studies showed that it is widely expressed in multiple tissues along with syncytiotrophoblasts and extravillous trophoblasts and is associated with the inflammation process and atherosclerosis [10,11]. PAPP-A has a prominent role in IGF system, reconfiguring some subgroups of insulin-like growth factor binding proteins (IGFBPs). It is strongly anabolic by IGF-dependent cellular effects [10]. Noteworthy, mouse models have shown that lifespan was increased in the absence of PAPP-A [12,13]. These findings indicate the detrimental effects of PAPP-A in IGF dependent inflammatory process. However, its potential atherogenic effects are not related with IGF-inflammation pathway. PAPP-A also promotes procoagulant activity by Akt-NF- κ B pathway in human endothelial cells [14]. In atherosclerotic plaques, PAPP-A was highly expressed and is related to increased risk of atherosclerotic plaques rupture [11].

IGF pathway plays crucial roles in follicular development including recruitment, apoptosis, growth, steroid hormone synthesis [15]. Eventually, altered physiology in PCOS is associated with long-term CVD risk. However, the literature is limited regarding PAPP-A levels in PCOS. Therefore, in this study, we aimed to investigate the serum PAPP-A levels among lean PCOS women as a cardiovascular risk marker.

Materials and methods

Subjects

This was a case-control study conducted at a university hospital setting. Ethical committee approval (PAU-GOKAEK, 29.12.20/76838) was obtained, and all participants gave informed consent before inclusion to the study. Young, non-obese women of reproductive age (18-35 years of age) who visited the hospital were invited to participate in the study. The Rotterdam criteria was used for the diagnosis of PCOS. Controls were recruited from healthy women and matched for age and body mass index (BMI). The control group consisted of women with regular menses who had normal ovarian morphology documented in the early follicular phase. Exclusion criteria were as follows: BMI \geq 27 kg/m², age \geq 35 years, diagnosed with a systemic disease such as cardiac disease, diabetes, liver disease, kidney disease etc., current, or up to 6 months of previous hormonal contraceptive use, a history of ovarian surgery.

Clinical data collection

Baseline characteristics, history, and BMI were recorded for each patient. The body mass index (BMI) was calculated as body weight (kg) / height (meters) squared. All subjects were evaluated by sonography using Voluson E 730 Pro (GE Healthcare, Istanbul, Turkey). Diagnosis of PCOS was achieved by the presence of at least two of the following criteria: (1) Clinical and/or biochemical hyperandrogenism, (2) Oligo-anovulation and (3) Ultrasonographic features of polycystic ovaries (PCO). Biochemical hyperandrogenism is defined by total and/or free testosterone levels above the upper limit or calculated free androgen index (FAI) $>$ 5. Oligo-anovulation was defined as menstrual cycles of \geq 35 or \leq 23 days or skipping at least three consecutive menstruation cycles.

Blood sampling and biochemical analysis

Serum samples were obtained after an overnight fast and analyzed immediately or centrifuged and stored until analysis at -20°C . PAPP-A was assayed using enzyme-linked immunosorbent assay (ELISA). Serum concentrations of SHBG, total testosterone, DHEAS, prolactin, and TSH were measured by electrochemiluminescent immunoassay with an inter and intra-assay coefficient of variation (CV) of $<$ 5%. Radioactive immunoassay method was used for the detection of serum levels of androstenedione and free testosterone. The plasma glucose levels were calculated by the hexokinase method. (HOMA-IR) was determined using the formula of homeostatic model assessment (HOMA) (fasting serum insulin [uIU/mL] x fasting plasma glucose [mmol/L]/22.5). Serum levels of Total cholesterol, high-density lipoprotein (HDL), and triglycerides were found using an enzymatic colorimetric assay. Analyses were performed on the Cobas e602 (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Data analysis was conducted using the R Statistical Computing software version 3.5.3 (Vienna, Austria), with R commander 2.6 package and SPSS 13 (SPSS, Inc., Chicago, IL) and SPSS 20 package (SPSS, Inc., Chicago, IL). Continuous variables were first examined by both visual inspection and the Shapiro-Wilk test. Normally distributed variables were evaluated using a Student t-test, while non-normally distributed continuous

variables were compared by Mann-Whitney U Test. Quantitative variables were expressed as mean (SD).

The sample size in each group was calculated as 40 (G*Power 3.1). This sample size yielded 90% power with an alpha error of 0.05 for detecting an effect size of 0.75 for the difference in PAPP-A measurement in women with a BMI below 27 kg/m², according to the previous report of Ozturk et al. [16].

Correlation was performed by Bivariate Pearson’s test between parameters. A multiple linear regression model was built to perform multivariable analysis for exploring independent factors related with PAPP-A levels. All parameters were presented as mean (SD) or frequency (%). Statistical significance level was set at $P < 0.05$.

Results

A total of 41 PCOS women and 40 age- and BMI-matched controls were analyzed. According to inclusion criteria all women had BMIs below 27 kg/m². Basic characteristics of the participants are summarized in Table 1. Women with PCOS had significantly higher levels of AMH (10.0 (5.1) ug/l vs. 4.5 (2.5) ug/l, $P < 0.001$), free testosterone (2.7 (1.2) ng/l vs. 1.9 (0.7) ng/l, $P = 0.001$), SHBG (43.7 (19.6) nmol/l vs. 53.1 (19.3) nmol/l, $P = 0.035$), total testosterone (0.5 (0.2) ug/l vs. 0.3 (0.1) ug/l, $P = 0.001$), free androgen index (FAI) (5.1 (3.5) vs. 3.0 (2.9), $P = 0.009$), and LH/FSH ratio (2.2 (1.3) vs. 1.2 (0.9), $P = 0.001$) when compared to controls (Table 2).

Table 1: Basic characteristics metabolic parameters of the study population

	Control (n=40)	PCOS (n=41)	P-value
Age (years)	24.3 (4.2)	23.1 (3.2)	0.134
BMI (kg/m ²)	22.3 (2.8)	22.2 (2.7)	0.953
Fasting glucose (mg/dl)	90.3 (7.3)	89.5 (6.4)	0.616
HOMA-IR	2.1 (1.3)	2.6 (2.1)	0.304
TC (mg/dl)	176.5 (28.6)	172.5 (27.0)	0.527
LDL (mg/dl)	98.4 (25.5)	95.8 (26.2)	0.665
HDL (mg/dl)	62.9 (16.3)	58.7 (15.6)	0.252
TG (mg/dl)	76.0 (33.5)	92.1 (40.3)	0.062

PCOS: polycystic ovarian syndrome, BMI: body mass index, AMH: anti Mullerian hormone, HOMA-IR: homeostatic model assessment of insulin resistance, TC: total cholesterol, HDL: high-density lipoprotein, LDL: low-density lipoprotein, TG: Triglycerides

Table 2: Hormonal parameters of the study population

	Control (n=40)	PCOS (n=41)	P-value
AMH (ug/l)	4.5 (2.5)	10.0 (5.1)	<0.001
Free testosterone (ng/l)	1.9 (0.7)	2.7 (1.2)	0.001
SHBG (nmol/l)	53.1 (19.3)	43.7 (19.6)	0.035
Total testosterone (ug/l)	0.3 (0.1)	0.5 (0.2)	0.001
Free Androgen Index	3.0 (2.9)	5.1 (3.5)	0.009
DHEA-S (ug/dl)	280.0 (94.9)	297.4 (117.6)	0.492
Androstenedione (ng/ml)	2.1 (0.9)	2.6 (1.5)	0.122
LH/FSH ratio	1.2 (0.9)	2.2 (1.3)	0.001

PCOS: polycystic ovarian syndrome, AMH: anti Mullerian hormone, SHBG: sex hormone binding globulin, DHEAS: dehydroepiandrosterone sulfate, LH/FSH: luteinizing hormone/ follicle-stimulating hormone

The control and PCOS groups were comparable in terms of serum PAPP-A levels (1.82 (0.66) ng/m vs. 2.03 (0.63) ng/m, $P = 0.128$). We further compared PAPP-A levels after defining PCOS subgroups according to the presence of HA and oligo-anovulation. PCOS women with and without HA had similar serum PAPP-A levels (2.0 (0.7) ng/m vs. 2.2 (0.6) ng/m, $P = 261$). Serum PAPP-A was higher in the PCOS women with oligo-anovulation compared to the control group (2.3 (0.5) ng/m vs. 1.8 (0.7) ng/m, $P = 0.006$). However, PCOS women without oligo-anovulation had comparable PAPP-A levels with the control group (1.7 (0.6) ng/m vs. 1.8 (0.7) ng/m, $P = 0.613$) (Figure 1).

Serum PAPP-A levels were not significantly correlated with age ($r = -0.117$, $P = 0.300$) or BMI ($r = -0.002$, $P = 0.989$)

among the study population. PCOS women were also separately analyzed for the correlation of PAPP-A with metabolic and hormonal parameters; however, none of these comparisons yielded significant results. We further performed a multiple linear regression analysis for age, BMI, oligo-anovulation, FAI and AMH as independent parameters to predict PAPP-A levels. This analysis revealed oligo-anovulation to be a predictor of serum PAPP-A levels even after controlling for age, BMI, FAI and AMH, which may have confounding effects. Table 3 summarizes the results of the multiple linear regression model for predicting PAPP-A levels.

Figure 1: Comparison of serum PAPP-A levels (OA: oligoanovulation, * $P = 0.003$, ** $P = 0.006$)

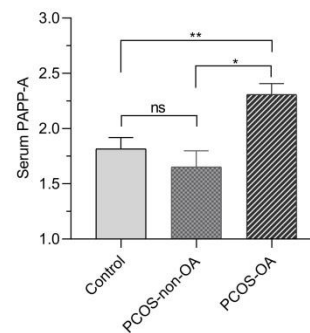


Table 3: Multiple regression model for predicting PAPP-A levels

	Unstandardized coefficient	95% CI		t statistics	P-value
		Minimum	Maximum		
Oligo-anovulation	0.509	0.132	0.887	2.697	0.009
Age	-0.036	-0.094	0.022	-1.245	0.218
BMI	0.005	-0.054	0.064	0.161	0.872
AMH	-0.012	-0.050	0.027	-0.617	0.540
FAI	0.037	-0.015	0.088	1.417	0.162

CI: confidence interval, BMI: body mass index, AMH: anti Mullerian hormone, FAI: free androgen index

Discussion

The aim of the present study was to compare serum levels between PCOS and controls and analyze whether any other variables were associated with PAPP-A levels. For this purpose, a case control study was conducted among 81 age- and BMI- matched non-obese young women. Overall, no difference in PAPP-A levels were observed between the PCOS and control groups. However, when PCOS women were further analyzed in subgroups of phenotypes, serum PAPP-A levels were higher in women with PCOS who had oligo-anovulation than the control group and PCOS patients without oligo-anovulation. A multiple regression analysis also revealed oligo-anovulation to be a predictor of serum PAPP-A levels even after controlling for the confounding effects of age, BMI, FAI and AMH. In other words, presence of oligo-anovulation per se was associated with increased PAPP-A levels when all other factors were controlled.

Öztürk et al. [16] evaluated the serum levels of PAPP-A in patients with PCOS, age- and BMI -matched controls. They found no significant difference of median serum levels of PAPP-A (1.7 ng/ml versus 1.8 ng/ml, respectively, $P = 0.328$) between PCOS patients and controls. However, a subgroup analyses related to BMI revealed that among women with BMI <27 kg/m², patients with PCOS exhibited higher PAPP-A levels than controls (2.1 ng/ml versus 1.8 ng/ml, respectively, $P = 0.018$). When women with PCOS were investigated among themselves in terms of BMI, lean PCOS cases had higher levels of PAPP-A (2.1 ng/ml versus 1.5 ng/ml, $P = 0.002$). PAPP-A levels were also

negatively correlated with age, BMI, and triglyceride levels. The authors concluded that PAPP-A might be a clinical indicator for the cardiovascular risk evaluation in a subgroup of young patients with BMI <27 kg/m² using PAPP-A. Apart from cardiovascular risk, these results also suggested that increased PAPP-A levels may be associated with abnormal folliculogenesis in lean PCOS groups due to higher IGF activity independent of excess fat tissue. Since IGF system has a significant role in FSH resistance at the granulosa level and androgen-dominant milieu in PCOS, this topic needs further evaluation.

In our study, the patients with PCOS and control group were comparable regarding their HOMA levels, which prevented the possible bias related with the increase in cardiovascular risk associated with metabolic syndrome and insulin resistance. Our findings were interesting for demonstrating high serum PAPP-A levels in PCOS patients with oligo-anovulation. Since this was a case control study, our results were not conclusive in defining the direction of causality for the correlation between increased levels of PAPP-A and oligo-anovulation. Further investigations are needed including tissue levels of PAPP-A in this subgroup of women.

Due to the unfavorable cardio-metabolic profile, women with PCOS are considered at increased risk for cardiovascular disease (CVD). In fact, previous studies exploring subclinical atherosclerosis showed increased carotid-intima media thickness and arterial stiffness, and impaired flow mediated dilatation [4,17,18]. Moreover, several large cohort studies confirmed that these women are at increased risk for CVD [19,20]. However, some authors consider that cardiovascular risk in these patients are related to traditional risk factors of obesity, hypertension, or dyslipidemia [21]. On the other hand, PCOS is a heterogeneous disorder with substantial variations between the phenotypes [22,23]. Distinct mechanisms can mediate the cardiovascular risk in different phenotypes. Hormonal disturbances or chronic oligo-anovulation that eventually result in disturbed hormonal milieu can be one of these possible alterations. In this study, we found that ovulatory abnormalities were associated with increased serum PAPP-A levels, independent of other classical CVD risk factors.

During the follow-up and management of PCOS patients, the heterogeneous nature of the disease can sometimes be ignored. Insufficient consultation on the comorbidities and long-term risks of the disease is not uncommon. There is remarkable clinical variation in the presentation of the syndrome throughout life. Patients with PCOS may experience considerable additional cardiovascular comorbidities along with advanced age. The American College of Obstetricians and Gynecologists [24] emphasizes that BMI, fasting lipid profile, and metabolic syndrome components should be evaluated in all PCOS cases. However, there is a need for defining novel markers to determine the risk of cardiovascular disease in distinct PCOS phenotypes. Our results indicated that oligo-anovulation may be independently associated with an increase in long-term cardiovascular risk. One interesting point of this finding is that the PAPP-A levels of PCOS patients without oligo-anovulation was similar to those of controls. Further prospective studies are needed to define additional cardiovascular disease risk markers in this subgroup of women.

Consuegra-Sanchez et al. [11] reviewed currently available data about PAPP-A to assess its predictivity for CVD. The authors also discussed some of the criticisms regarding the value of this marker in clinical practice. They concluded that PAPP-A may have a role atherosclerotic lesion development and may relate to the instability of the atheromatous plaque. However, the exact mechanism of PAPP-A in cardiovascular disease pathogenesis is yet to be explained. Papanastasiou et al. [25] conducted a systematic review of the current literature to determine the prognostic value of PAPP-A for cardiovascular events among patients presenting with chest pain. The authors investigated 8 studies according to the inclusion criteria. One important limitation of this review was the use of different assays for circulating PAPP-A measurements resulting in heterogeneity in cut-off values and patient stratification. However, it did not restrain the meta-analytic approach to quantitatively synthesize research findings for each of the articles. This meta-analysis supported that PAPP-A levels were predictive for especially early complications of cardiovascular events. The authors proposed that it may be related to fluctuation of the inflammation over time. However, there is still lack of prospective studies to draw conclusions for the prognostic value of PAPP-A in women with PCOS.

Strength and limitations

There are two major strengths in this study. First, we analyzed a homogeneous group of PCOS women who were strictly evaluated according to Rotterdam criteria. Second, we excluded obese women, which allowed us to avoid the confounding effects of obesity. Our results indicate that PAPP-A levels holds promise for cardiovascular risk stratification in distinct PCOS phenotypes. To our knowledge, this is the first study demonstrating a positive association between chronic oligo-anovulation and PAPP-A, which is also a marker for cardiovascular risk. The main limitation of this study was its case control design that precludes us from determining the direction of the association between oligo-anovulation and elevated PAPP-A levels. Second, the relationship between PAPP-A levels and cardiac function was not explored. However, the homogeneous group of women recruited stands as a strength for eliminating the confounding effects of insulin resistance and obesity. There can be several sources of bias in this study: First, selecting a lean group (BMI < 27 kg/m²) may limit the generalization of these results to entire population, and second, diagnosis of PCOS may raise discrepancies when different diagnostic criteria are used. These should be considered when comparing these results with others. Prospective studies are needed to uncover the long-term cardiovascular risk of elevated PAPP-A levels in PCOS patients with oligo-anovulation.

Conclusions

Serum PAPP-A levels are similar in the control and PCOS groups. However, lean, and young PCOS patients with oligo-anovulation have increased serum levels of PAPP-A when compared to women without. This association is independent of the confounding effects of age, BMI, FAI and AMH.

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