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Original Article

Investigation of Serum PAPP-A, IGF-II, and IGFBP-4 Levels in Patients with Polycystic Ovary Syndrome - A Prospective Case-Control Study

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Abstract

Objective: *The etiology of polycystic ovary syndrome involves complex interactions. Defining new parameters will speed up the diagnostic process. Pregnancy-associated plasma Protein A, Insulin-like Growth Factor II, and Insulin-like Growth Factor Binding Protein 4 are candidates for becoming new parameters of the diagnostic process. The study aims to investigate the role of these parameters in the etiopathogenesis of polycystic ovary syndrome.*

Methods: *The study was conducted on volunteer women of reproductive age who met the Rotterdam diagnostic criteria. A total of 87 participants, consisting of 45 patients and 42 healthy controls, were included in the study. The present study is a prospective, single-center study. The patients' hirsutism scores were determined with the modified Ferriman-Gallwey scoring system. Ovaries were evaluated, and Antral Follicle Count measurements were taken. Descriptive statistics were presented. Independent risk factors were investigated through Logistic Regression Analyses.*

Results: *It was observed that the antral follicle count, Pregnancy-Associated Plasma Protein A, and Insulin-like Growth Factor Binding Protein 4 values were significantly elevated in the PCOS group. In the polycystic ovary syndrome cohort, Pregnancy-Associated Plasma Protein A values demonstrated a significant positive correlation with FSH and HbA1c. The inclusion of the hirsutism score as a variable demonstrated its significance (p=<0.001; OR=19.173, 95% CI: 6.101-60.251), whereas potential marker levels were not independently associated with polycystic ovary syndrome risk.*

Conclusion: *Although some measurements between the groups differed in terms of potential markers, the effectiveness of these three parameters did not reach the level of the hirsutism score.*

Introduction

Polycystic Ovary Syndrome (PCOS) represents a complex endocrine disorder predominantly affecting women of reproductive age. The etiology of PCOS remains multifaceted, involving intricate interplays between genetic predispositions, environmental factors, and lifestyle influences [1]. Emerging research continues to elucidate the underlying mechanisms of PCOS, promising advancements in diagnostic accuracy and therapeutic interventions [2].

Biochemical assays play a pivotal role, emphasizing measuring serum levels of androgens, including testosterone, androstenedione, and dehydroepiandrosterone sulfate. Evaluating the lipid profile, glucose tolerance, and insulin resistance parameters further enriches the diagnostic process, given the association of PCOS with metabolic syndrome and increased risk of type 2 diabetes mellitus [3] The delineation of new parameters will enrich the diagnostic process and expedite its pace.

The biomarker, Pregnancy-Associated Plasma Protein A (PAPP-A), plays a pivotal role in prenatal screening and diagnosis. It is a glycoprotein synthesized by placental trophoblasts. Ongoing research elucidates its interactions with other biochemical and physiological parameters, underscoring its potential to enhance maternal-fetal health outcomes [4].

Insulin-like Growth Factor II (IGF-II) is a pivotal component in cellular growth, differentiation, and metabolism [5]. In the realm of gynecology, the effects of IGF-II are multifaceted, encompassing ovarian function,

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placental development, and patterns of fetal growth [5]. Current research is increasingly concentrated on elucidating the versatile roles of IGF-II in gynecology, underscoring its significance in this domain [6].

Insulin-like Growth Factor Binding Protein 4 (IGFBP-4) constitutes a pivotal element within the intricate regulatory network governing reproductive physiology, exerting extensive effects in gynecology [7]. This protein is an integral component of IGF modulation [7]. Recent advancements have elucidated the role of IGFBP-4 in the pathophysiology of PCOS. Research focusing on IGFBP-4 harbors the potential to foster the development of novel therapeutic strategies for treating gynecological disorders [8].

This study aims to investigate the role of PAPP-A, IGF-II, and IGFBP-4 in the etiopathogenesis of PCOS and to determine their levels in individuals with PCOS, subsequently comparing these findings with those of healthy subjects.

Material & methods

Study Population

The study was conducted between January and October 2014 at a Training and Research Hospital's Department of Obstetrics and Gynecology in Turkey. It was conducted among volunteer women of reproductive age (18-40 years) who met the Rotterdam diagnostic criteria [9]. A total of 87 participants were included in the study, comprising 45 patients diagnosed with PCOS and 42 healthy controls confirmed to have regular menstrual cycles and ovulatory function. Participants in the control group are within the age range of 18 to 40 years, exhibit no clinical or biochemical hyperandrogenism, and possess body mass indices comparable to those of the patient group.

Power Analysis

The sample size was determined through power analysis. The effect size was established at d=0.75, with a power (1-β) of 0.90, and an allocation ratio of 1 was assumed. Within this framework, the minimum sample size was at least 39.

Study Design and Participants

This is a prospective, single-center study. The diagnosis of PCOS is established upon the presence of at least two criteria from the Rotterdam guidelines. The clinical presence of oligo-amenorrhea determined oligo-anovulation. The clinical indicators of hyperandrogenism were primarily based on the presence of hirsutism or acne. The hirsutism scores of the patients were ascertained using the modified Ferriman-Gallwey (mFG) scoring system [10]. According to this system, hair density in nine anatomical regions—including the upper lip, chin, chest area, back, abdomen, upper and lower abdomen, arms, and upper legs—was rated on a scale from 0 to 4. Individuals with a total score of eight or higher were considered hirsute. In ultrasonography, enlarged ovaries due to increased stromal tissue and the appearance of polycystic ovaries were characterized by 12 or more follicles measuring 2-8 mm in size, distributed in a 'pearl necklace' pattern around the periphery.

The examination of participants commenced with a narrative and physical assessment. The inquiry covered the ages of the patients, numbers of pregnancies, births, and miscarriages, gynecological histories, past surgeries, presence of systemic diseases, suspicion of bleeding diatheses or thrombosis, and use of tobacco, alcohol, or narcotics. Body Mass Index (BMI) values of participants were calculated. Waist circumference was measured at the level of the umbilicus, based on the significant trochanter, and hip circumference was also assessed. Waist-to-hip ratios were computed.

Ultrasonographic and hormonal evaluations of the participants were conducted between the second and fifth days of menstruation. The ultrasonographic examination was performed using a MINDRAY brand DC-7T model ultrasound machine, transvaginally in the lithotomy position. Ovaries were evaluated, and Antral Follicle Count (AFC) measurements were taken. During the ultrasonographic examination, 12 or more follicles ranging in diameter from 2-8 mm indicated a polycystic ovarian morphology according to the Rotterdam PCOS diagnostic criteria.

Samples from participants who did not experience menstruation at the time of their initial consultation and from other participants during the second to fifth days of menstruation were collected.. Two venous blood samples, each 2 milliliters, were collected following a minimum fasting period of 12 hours. These samples were centrifuged at 4000 rpm for 10 minutes for the analysis of PAPP-A, IGF-II, and IGFBP-4 and subsequently stored at -80°C until the day of analysis.

Inclusion Criteria

- Voluntary participation,
- Regular menstrual cycle (control group),
- The absence of clinical or biochemical hyperandrogenism (control group)

Exclusion Criteria

- Patients with endocrinopathies, including diabetes mellitus, thyroid dysfunction, Cushing's syndrome, tumors secreting androgens, and late-onset 21-hydroxylase deficiency,
- Infectious diseases,
- Hypertension,
- Hyperprolactinemia,
- Chronic liver disease,
- In the preceding six-month period, the utilization of pharmacological agents that modulate the profile of sex hormones, including oral contraceptives, anti-androgens, and treatments for infertility,
- The employment of medications that impact the metabolism of carbohydrates and lipids,
- Ovarian pathologies, such as endometriomas, dermoid cysts, or simple cysts, adversely affect ovarian functions,
- History of ovarian surgery,
- Consumption of alcohol and tobacco.

Serum analyses

In the early follicular phase of the menstrual cycle, a 5 ml blood sample was procured to quantify levels of total cholesterol, triglycerides, HDL, LDL, and VLDL. The concentrations of fasting blood glucose, basal insulin, FSH, LH, TSH, estradiol, total testosterone, DHEAS, androstenedione, SHBG, and 17-hydroxyprogesterone were analyzed using the chemiluminescence method on a Beckman Coulter DXI 800 (Beckman Coulter Istanbul/TURKEY) instrument. The levels of HbA1c were examined via HPLC. The formula fasting plasma insulin (mIU/L) x fasting plasma glucose (mmol/L) / 22.5 was utilized to determine insulin resistance. Values equal to or exceeding 2.5 were considered indicative of insulin resistance. Serum levels of PAPP-A were analyzed using the chemiluminescent ELISA technique on the Beckman Coulter DXI 800 apparatus. Concurrently, serum levels of IGF-II and IGFBP-4 were assessed utilizing the ETI-max 3000 Human ELISA Kit (TURKLAB Ankara/TURKEY).

Ethics

The study has been designed by the Declaration of Helsinki. Authorization was obtained from the institution's ethics committee where the research was conducted (Ethics Committee Decision Date - No: 12.12.2013- 30/10). Participants were informed in writing and verbally before inclusion in the study, and their consent was secured.

Statistical Analysis

The analyses were conducted utilizing the SPSS 18.0 software suite. Descriptive statistics were presented, including frequency, percentage, mean, standard deviation (SD), median, minimum, and maximum values. The relationships between categorical variables were examined using Fisher's Exact or Pearson's chi-square tests. The conformity to normal distribution was assessed by employing Shapiro-Wilks and Kolmogorov-Smirnov tests. The Mann-Whitney U test and Student's t-test were applied to analyze the differences between the two groups. Spearman's correlation analysis was utilized to explore the relationships among continuous variables. Receiver Operating Characteristic (ROC) analysis was conducted to differentiate patients with PCOS based on specific measurements and to establish cut-off points. Cut-off points, sensitivity, and specificity values were provided for all markers. Independent risk factors were investigated through Logistic Regression Analysis. Results were presented with p-values, odds ratios, and 95% confidence intervals. P-values less than 0.05 were considered statistically.

Results

The socio-demographic characteristics of the participants have been scrutinized. The average age is 28.60 ± 4.30 in PCOS and 28.57 ± 6.22 control groups. No significant difference has been found between the groups regarding their family histories. In 44.4% of the participants with Polycystic Ovary Syndrome (PCOS), all three Rotterdam criteria are present. The age, Body Mass Index (BMI), gravida, and parity values of both groups are similar (p>0.05). The waist-to-hip ratios of the patients with PCOS are significantly higher than those of the control group (p=0.002).

The clinical and laboratory findings of the participants were compared. Accordingly, it was observed that the AFC mFG score, LH, total testosterone, total cholesterol, LDL, PAPP-A, and IGFBP-4 values were significantly elevated in the PCOS group, whereas the SHBG value was diminished. No difference was found in the other measurements between the two groups (Table 1).

In the PCOS cohort, PAPP-A values demonstrated a significant positive correlation with FSH and HbA1c (Figure 1), whereas in the control group, a meaningful positive association was observed with AFC, hirsutism score, and FSH (Figure 2). IGF-II exhibited a significant, albeit modest, negative relationship with glucose levels exclusively within the control group (p=0.013). No significant correlation was identified between IGFBP-4 and any variable under investigation (p>0.05).

Table 1. *Clinical and laboratory findings of the participants*

	PCOS Mean±SD	**Control Mean±SD**	**p**
AFC	20,02±9,34	7,96±2,06	<0,001
Hirsutism Score	14,11±7,55	6,17±4,04	<0,001
FSH	5,67±1,72	5,78±1,84	0,768
LH	8,19±4,76	4,65±3,23	<0,001
Estrogen	65,78±83,90	72,79±123,30	0,929
TSH	1,62±0,86	1,43±0,89	0,216
Prolactin	12,27±7,14	10,43±4,55	0,400
17-OH Progesterone	1,55±1,14	1,21±0,41	0,281
DHEAS	233,05±107,73	233,04±113,11	0,899
SHBG	54,20±48,36	59,87±40,09	0,035
Total testosterone	0,50±0,21	0,38±0,16	0,007
HbA1c	5,34±0,35	5,29±0,33	0,223
Total Cholesterol	185,24±42,71	165,50±33,65	0,021
Triglycerides	104,51±61,66	87,71±39,90	0,253
LDL	110,56±35,21	89,74±29,07	0,002
VLDL	20,64±12,37	17,64±7,96	0,383
HDL	52,80±13,81	60,67±26,46	0,257
Insülin	8,77±6,01	9,17±6,20	0,899
Glucose	87,89±9,35	85,76±8,78	0,278
HOMA	1,93±0,97	1,92±1,07	0,677
PAPP-A	1,70±0,39	1,53±0,43	0,029
IGF-II	330,05±557,71	182,25±251,60	0,112
IGFBP-4	14,78±8,98	11,52±7,63	0,047

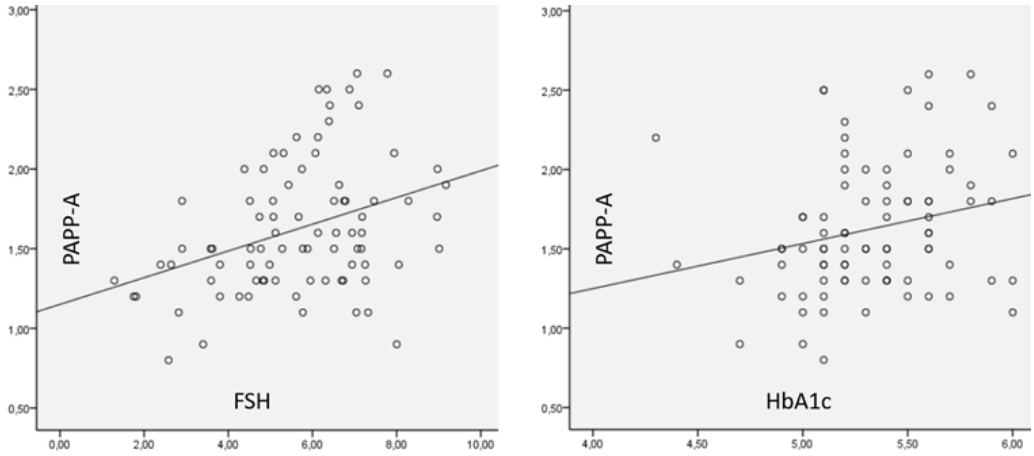

Figure 1. *A correlation analysis graph in PCOS group*
a. Between PAPP-A and FSH
b. Between PAPP-A and HbA1c.

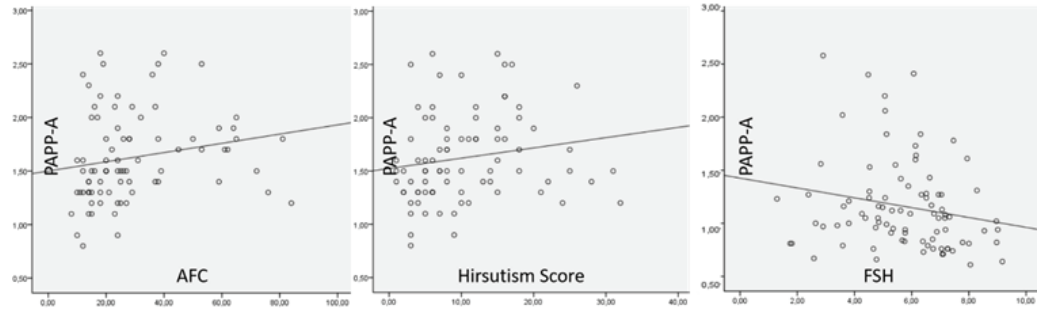

Figure 2. *A correlation analysis graph in control group.*
a. Between PAPP-A and AFC
b. Between PAPP-A and Hirsutism Score.
c. Between PAPP-A and FSH.

Table 2. *Binary Logistic Regression Analysis.*

	OR	p	OR %95 Lower Limit	OR %95 Upper Limit
Model 1				
PAPP-A	2,591	0,097	0,841	7,982
IGFBP-4	1,037	0,209	0,980	1,096
Model 2				
PAPP-A cut-off	2,007	0,274	0,576	6,991
IGFBP-4 cut-off	2,835	0,037	1,063	7,564
Model 3				
Hirsutism score	19,173	<,001	6,101	60,251
PAPP-A cut-off	2,075	0,359	0,435	9,891
IGFBP-4 cut-off	2,207	0,211	0,638	7,635

A ROC analysis was conducted to discern PCOS with greater precision. The optimal points for sensitivity and specificity were identified as>1.3 for PAPP-A (sensitivity=84.4; specificity=44.7) and >8.6 for IGFBP-4 (sensitivity=80; specificity=45.2). These thresholds were subsequently utilized in a multivariate logistic regression analysis.

In the context of PCOS, 38 patients (84.4%) exhibited a hirsutism score exceeding 8, compared to merely nine patients (21.4%) in the control group. This disparity between the groups is statistically significant (p<0.001). In the Binary Logistic Regression model, Model 1 incorporated only PAPP-A and IGFBP-4 measurements, revealing that these variables did not independently predict PCOS risk (p>0.05). However, in a subsequent model that also considered the threshold levels of these measurements, only IGFBP-4 values exceeding 8,6 were identified as an independent risk factor for PCOS (p=0.037; OR=2.835, 95% CI: 1.063-7.564). In the final model, the inclusion of the hirsutism score as a variable demonstrated its significance (p=<0.001; OR=19.173, 95% CI: 6.101-60.251), whereas PAPP-A and IGFBP-4 levels were not independently associated with PCOS risk (Table 2).

In conclusion, although there exists a discernible disparity in the measurements of PAPP-A and IGFBP-4 between groups when potential markers for PCOS were scrutinized, it has been observed that the efficacy of these three indicators did not attain the level of success as demonstrated by the hirsutism score.

Discussion

The present study evaluated PAPP-A, IGF-2, and IGFBP-4 as potential markers. Although there exists a discernible disparity in the measurements of PAPP-A and IGFBP-4 between groups, the efficacy of these three indicators did not attain the level of success as demonstrated by the hirsutism score. PCOS imposes a substantial burden on metabolic homeostasis, predisposing affected individuals to an elevated risk of obesity, insulin resistance, type 2 diabetes mellitus, dyslipidemia, cardiovascular diseases, non-alcoholic fatty liver disease, and the symptoms of anxiety, depression, and diminished quality of life [3,11-14]. Considering PCOS as a systemic disease that causes metabolic comorbidities, the search for a biomarker that can diagnose this disease earlier continues today [14].

In our study, the waist-hip circumference ratio was significantly higher in patients with PCOS compared to the control group. A ratio exceeding 0.85 indicates android obesity, a condition characterized by excessive fat accumulation in the abdominal area [15]. This ratio serves as a crucial parameter in reflecting the quantity of body fat. Extensive literature exists indicating that individuals with PCOS typically exhibit a waist-to-hip circumference ratio that surpasses the norm [11,15]. Our findings are in concordance with this documented evidence. However, our research does not support the waist-to-hip ratio as a sufficient diagnostic criterion for android obesity in the PCOS cohort. Notwithstanding, the significant elevation in total cholesterol and LDL levels can be attributed to the increased waist-hip ratios observed, suggesting a potential linkage between these metabolic parameters and the distribution of body fat.

Hirsutism refers to the manifestation of terminal hair in females exhibiting a distribution pattern typically observed in males, affecting approximately 5-10% of women [12]. It serves as the primary indication of hyperandrogenism in PCOS, with a prevalence rate of roughly 60% [13]. The mFG scoring system is employed to visually assess and score the extent of excessive terminal hair, providing a standard for evaluation and facilitating comparison. There is no universal cutoff point for the mFG score [16]. In the assessment of hirsutism, ethnic background and dermatological characteristics should be taken into consideration. Studies have demonstrated a weak correlation between the severity of hirsutism and androgen levels [17]. Although serum androgen concentrations in patients with hirsutism associated with PCOS are frequently elevated beyond reference values, a subset of these individuals does not exhibit biochemical hyperandrogenism. This complicates the diagnosis of PCOS.

Based on the outcomes of our research, the hirsutism score emerges as a parameter of superior value in diagnosing PCOS compared to other criteria. According to the binary logistic regression analysis results, the hirsutism score could be statistically significant as a diagnostic criterion.

In a study comparing levels of PAPP-A between women diagnosed with PCOS and control subjects, it was determined that serum PAPP-A levels exhibited similarity across both cohorts [18]. However, when comparisons were adjusted for BMI, individuals with PCOS demonstrated elevated levels of PAPP-A. A negative correlation was identified between PAPP-A levels and several parameters, including age, BMI, and triglyceride levels. This led to the conclusion that PAPP-A could be a clinical marker in PCOS. It is assessed that individuals with a lower BMI might benefit from risk assessment procedures utilizing PAPP-A levels. The outcomes of our investigation do not endorse the utilization of PAPP-A as a viable diagnostic tool for PCOS.

Another study's outcomes align with our research [19]. Accordingly, the levels of human chorionic gonadotropin, PAPP-A, and Estriol in women with PCOS are comparable to those in the control group. The study's participant count surpasses ours, and its design is similar. From this perspective, the parallel nature of the findings is significant.

Numerous observations have elucidated the pivotal role of IGF-2 in the pathogenesis of PCOS [20,21]. There is substantial evidence to suggest that IGF-II disrupts folliculogenesis. In PCOS, the levels of IGF-1 and IGF-2 within antral follicles are normal. However, within the granulosa cells of dominant follicles, there is an upsurge in the expression of IGF-2, while IGF-I is undetectable [22]. Moreover, in women afflicted with PCOS, the concentration of IGF-II in follicular fluid is markedly elevated when compared to controls [22]. Hyperinsulinemia in PCOS patients suppresses the hepatic production of IGFBP-1, concurrently leading to an increase in IGF-2 [23]. Variants within the IGF-2 gene cluster are associated with the diverse metabolic phenotypes observed in PCOS [24]. These variants have the potential to trigger obesity, metabolic syndrome, and cardiovascular risk factors inherent in PCOS.

Genetic variants may augment susceptibility to hyperandrogenism. The G alleles of the ApaI variant, associated with IGF-2 expression, enhance androgen secretion. Patients with PCOS are homologous for the alleles of this variant. Consequently, targeting IGF-II to diminish hyperandrogenism could enhance fertility [25].

IGFBPs have been identified in the follicular fluid of patients diagnosed with PCOS. Compared to the follicular fluid from healthy follicles, IGFBP 2 and 3 concentrations are notably elevated in individuals afflicted with PCOS. However, it is worth noting that IGFBP-1 was not detected at significant levels. Furthermore, it is articulated that specific isoforms of IGFBP-4 may also experience an upsurge in patients suffering

from PCOS [26].

In another study, a comparative analysis of the cellular expression of IGFBP-4 between the ovaries of women with and without PCOS was conducted, revealing a significant disparity in those afflicted with PCOS [27,28]. Given the high affinity of IGFBP-4 for IGF-1, it has been emphasized that the augmented expression of IGFBP-4 in the ovaries of individuals with PCOS could diminish the bioavailability of IGF-I. This process disrupts the induction of aromatase activity, potentially fostering the development of the androgenic microenvironment characteristic of PCOS follicles.

This comprehensive analysis underscores the critical role of specific biomarkers and clinical features in the nuanced diagnosis of PCOS, highlighting the utility of sophisticated statistical models in elucidating the complex interrelations inherent in gynecological pathologies.

Conclusions

Our study possesses distinct strengths and limitations. Foremost among its strengths is its exceptionally well-structured design. Advanced statistical analyses have been utilized in the study. Support from relevant fields of expertise was sought for the laboratory work. The number of participants is sufficient to lend reliability to the study's outcomes. One of the limitations is the study's design as a case-control study. From the perspective of evidence-based medicine, cohort studies yield more reliable results than case-control. Therefore, this subject could be further investigated in future cohort studies.

Author Contributions

MKO: Protocol/project development, Data collection, Data analysis, Manuscript writing/editing. MKO & OE & GAB: Protocol/project development, Manuscript editing. MKO & HYE & MK: Data collection, Data analysis..

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Declarations

Conflict of interest: There are no conflicts of interest or disclosures.

Ethical approval: Our study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics committee permissions were obtained from the institution where the study was conducted. Ethics Committee Decision Date and Number: 12.12.2013- 30/10).

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