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POLISH GYNECOLOGY
GINEKOLOGIA POLSKA
ORGAN POLSKIEGO TOWARZYSTWA GINEKOLOGICZNEGO
THE OFFICIAL JOURNAL OF THE POLISH GYNECOLOGICAL SOCIETY

ISSN: 0017-0011

e-ISSN: 2543-6767

Unmasking metabolic clues: adipsin, irisin and osteopontin as biomarkers in polycystic ovary syndrome and their impact on metabolic dynamics: a case-control study

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DOI: 10.5603/gpl.102676

Article type: Research paper

Submitted: 2024-09-19

Accepted: 2025-01-10

Published online: 2025-03-05

ORIGINAL PAPER / GYNECOLOGY

Unmasking metabolic clues: adipsin, irisin and osteopontin as biomarkers in polycystic ovary syndrome and their impact on metabolic dynamics: a case-control study

Short title: Biomarkers in PCOS: adipsin, irisin, osteopontin

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ABSTRACT

Objectives: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, often associated with metabolic alterations. This study assessed serum levels of adipsin, irisin, and osteopontin in patients with PCOS and examined their correlations with metabolic parameters.

Material and methods: A case-control study was conducted involving 96 women with PCOS and 80 healthy controls. Serum levels of adipsin, irisin, and osteopontin were measured; demographic, clinical, and metabolic characteristics were evaluated.

Results: Patients with PCOS were significantly younger than controls ($p < 0.001$). The PCOS group included a significantly greater proportion of obese individuals ($p = 0.013$). Patients with PCOS exhibited elevated serum adipsin ($p = 0.020$) and reduced osteopontin ($p < 0.001$) levels relative to controls; obesity and age influenced these differences. Osteopontin demonstrated superior predictive power for PCOS diagnosis [area under the curve (AUC) = 0.802] compared with adipsin (AUC = 0.602). A combination of osteopontin and adipsin yielded the highest predictive value (AUC = 0.817) among double or triple biomarker combinations.

Conclusions: This study identified potential associations among adipsin, osteopontin, irisin, and PCOS. Further research is warranted to elucidate their roles and clinical implications in PCOS and its metabolic alterations. The findings highlight the impact of age and obesity on these biomarkers and their relationships with PCOS, providing insight into the syndrome's complex pathophysiology.
Keywords: polycystic ovary syndrome; adipsin; osteopontin; irisin; adipokines

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex clinical condition associated with increased prevalences of type 2 diabetes, hypertension, and potentially cardiovascular diseases. It is the most common endocrine and metabolic disorder in premenopausal women [1, 2]. Although its pathophysiology remains poorly understood, various cytokines that modulate inflammation, insulin sensitivity, carbohydrate and lipid metabolism are believed to play roles [1, 3]. Elevated levels of several cytokines in patients with PCOS result in chronic low-grade inflammation [2, 4].

Androgen excess, obesity, abdominal adiposity, adipose tissue dysfunction, and insulin resistance are considered pivotal pathogenic mechanisms in PCOS [1, 2, 5]. In patients with PCOS, altered adipokine secretion associated with obesity and abdominal adiposity may regulate metabolic and reproductive functions, as well as inflammatory responses [1, 4, 6, 7]. Adipose tissue dysfunction has been proposed to contribute to the development of PCOS and its metabolic consequences. The adipokine adipsin, secreted by white adipose tissue, influences systemic energy homeostasis and is involved in maintaining beta-cell function and triglyceride synthesis [4]. Elevated adipsin levels have been linked to PCOS, increased cardiovascular risk, and metabolic disturbances [1, 4].

Myogenic secretory factors, such as myokines, have been associated with improved PCOS phenotypes, reduced disease severity, and slower disease progression, although their exact mechanisms of action remain unclear [8]. Irisin, a thermogenic adipokine/myokine hormone, facilitates the conversion of white adipose tissue to brown adipose tissue, thereby increasing whole-body energy expenditure during exercise [7–10]. In addition to its production by skeletal muscle, adipocytes, and the heart, irisin is also synthesized in the ovaries and endometrium [9, 11]. Currently, debate persists concerning the relationship between irisin levels and PCOS in humans [7, 12–14].

The extracellular matrix-associated phosphoprotein osteopontin has a role in recruiting macrophages to inflamed adipose tissue, thus contributing to chronic low-grade inflammation

[15, 16]. Consequently, the induction of inflammatory signaling via osteopontin may impair the differentiation and insulin sensitivity of primary adipocytes [16]. However, the association between osteopontin and PCOS appears to vary across selected populations [15–18].

Objectives

Few studies have examined serum levels of adipsin, irisin, and osteopontin in patients with PCOS. This study aimed to enhance understanding of these molecules by evaluating their levels in the same patient population and investigating correlations of adipsin, irisin, and osteopontin levels with metabolic changes in patients with PCOS.

MATERIAL AND METHODS

Study design

This case-control study included patients with PCOS who attended follow-up at the Department of Obstetrics and Gynecology, Faculty of Medicine, Duzce University, Duzce, Turkey, between June 2022 and June 2023. The study was approved by the ethics committee of Duzce University (reference no. 2022/110). All procedures adhered to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Patients

Adults aged 18 to 45 years with a diagnosis of PCOS based on the 2003 Rotterdam Criteria [2] were enrolled. The diagnosis of PCOS required the presence of at least two of the following three criteria: clinical or biochemical signs of hyperandrogenism; oligo- or anovulation [7]; and polycystic ovarian morphology as determined by transvaginal ultrasound [2, 4]. Exclusion criteria were irregular menstrual cycles, Cushing's disease, hyperprolactinemia, congenital adrenal hyperplasia, thyroid disorders requiring medical treatment, pregnancy, galactorrhea, breastfeeding, and comorbid conditions (diabetes mellitus, hypertension, congestive heart failure, chronic renal failure, liver diseases, dyslipidemia, autoimmune or chronic inflammatory diseases, malignancies, and acute infectious diseases within the preceding 2 weeks). The final study cohort comprised 96 patients with PCOS. The control group included 80 healthy women aged 18 to 45 years with regular menstrual cycles and no coexisting diseases; these women were randomly selected from outpatient admissions.

Variables and data collection

The demographic characteristics (age and educational status), obstetric history (gravidity and parity), and clinical features (smoking status, alcohol consumption, and comorbidities) of the participants in both groups were recorded. Weight (kg), height (cm), and waist and hip circumferences (cm) were measured during outpatient visits. Body mass index (BMI, kg/m^2) was calculated as weight (kg) divided by height squared (m^2). Based on BMI values, participants were categorized as obese (BMI ≥ 30 kg/m^2) or non-obese (BMI < 30 kg/m^2) [1].

Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest [4]. Hip circumference was measured at the widest point of the buttocks [7]. The waist-to-hip ratio was calculated accordingly [6]. Hirsutism was evaluated using the Modified Ferriman–Gallwey score; a score of ≥ 8 indicated hirsutism [4].

Laboratory investigations

After an overnight fast, blood samples were obtained from all participants on the second or third day of their menstrual cycle. Laboratory measurements included glucose, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, insulin, glycated hemoglobin (HbA_{1c}), C-reactive protein (CRP), follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), prolactin, total testosterone, and thyroid-stimulating hormone (TSH). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting serum insulin and fasting plasma glucose as previously described [16]. The triglyceride/glucose index was also calculated using the relevant measurements for each participant [19].

Serum adipsin levels were measured using an enzyme-linked immunosorbent assay (ELISA) (Elabscience, catalog no. E-EL-H6007, USA) with intra- and inter-assay coefficients of variation (CVs) of < 6% and < 8%, respectively, and a sensitivity of 0.47 ng/mL [4]. Irisin levels were measured using a sandwich ELISA (Elabscience, catalog no. E-EL-H6120) with intra-assay CVs of 5.8% and 6.7%, along with a sensitivity of 0.09 ng/mL [7]. Serum osteopontin levels were measured using a sandwich ELISA (Elabscience, catalog no. E-EL-H1347) with intra- and inter-assay CVs of < 4.1% and < 6.7% [16].

Statistical analysis

Continuous variables were summarized as the mean ± standard deviation or median and range, depending on the distribution. Categorical variables were presented as frequencies

and percentages. Normality was assessed using the Shapiro–Wilk, Kolmogorov–Smirnov, and Anderson–Darling tests.

The Pearson chi-square test was used for 2×2 tables with expected counts ≥ 5. Fisher's exact test was applied to tables with counts < 5, and the Fisher–Freeman–Halton test was used for R×C tables with counts < 5.

The independent samples *t*-test was utilized to compare two groups with normally distributed variables, whereas the Mann–Whitney U test was used for variables with non-normal distributions.

Spearman's Rho correlation was used to evaluate relationships between continuous variables with non-normal distributions.

Adipsin, osteopontin, and irisin were analyzed as independent variables to predict PCOS. Predictive performance was evaluated using the area under the receiver operating characteristic curve (AUC). Integrated discrimination improvement (IDI) and net reclassification improvement (NRI) metrics were used to compare prediction models. IDI assessed differences in prediction accuracy; continuous NRI evaluated improvements in risk classification. Analyses were conducted using Python 3.8 with sci-kit-learn 0.24 and matplotlib 3.4 for visualization.

Other statistical analyses were performed using Jamovi (v2.3.28) and JASP (v0.17.3). A p value < 0.05 was considered statistically significant.

RESULTS

The study included 96 patients with PCOS (median age: 22 years) and 80 controls (median age: 29 years). Patients with PCOS were significantly younger than controls ($p < 0.001$). Active smoking was more common among controls ($p = 0.022$). A significant difference in BMI was observed ($p = 0.039$), with a greater proportion of obese patients in the PCOS group ($p = 0.013$). The modified Ferriman–Gallwey score was significantly higher in the PCOS group, indicating increased hirsutism ($p < 0.001$). Other clinical and sociodemographic characteristics were similar between the groups ($p > 0.05$) (Tab. 1).

Significant differences were identified between the PCOS and control groups in fasting insulin ($p = 0.008$), HOMA-IR ($p = 0.030$), triglycerides ($p = 0.002$), triglyceride/glucose index ($p = 0.011$), FSH ($p < 0.001$), LH ($p = 0.003$), LH/FSH ratio ($p < 0.001$), and total testosterone ($p < 0.001$). Other laboratory parameters did not significantly differ between the groups ($p > 0.05$) (Tab. 2).

Serum irisin levels were similar between the groups ($p = 0.747$), whereas significant differences were observed in serum adipsin ($p = 0.020$) and osteopontin ($p < 0.001$) levels. Patients with PCOS had higher adipsin and lower osteopontin levels relative to controls (Tab. 2, Fig. 1A–C).

Among patients with PCOS, obese individuals had significantly higher serum osteopontin and irisin levels than non-obese individuals ($p = 0.040$ and $p < 0.001$, respectively) (Tab. 3). Adipsin levels were not significantly different between obese ($p = 0.105$) and non-obese ($p = 0.336$) individuals (Fig. 1D). In the control group, obesity did not significantly affect serum osteopontin ($p = 0.598$) or irisin ($p = 0.068$) levels (Fig. 1E–F).

After adjustments for age ($p = 0.430$) and obesity ($p = 0.826$), serum adipsin levels remained similar between the groups (Fig. 2A). Although obesity did not significantly influence serum osteopontin levels ($p = 0.331$), age significantly affected differences in serum osteopontin levels between the groups ($p < 0.001$) (Fig. 2B).

Receiver operating characteristic curve analysis indicated that adipsin levels > 16.03 had an AUC of 0.602, whereas osteopontin levels ≤ 2.27 had an AUC of 0.802 for predicting PCOS (Fig. 3). Osteopontin demonstrated greater sensitivity (71.87%) and specificity (76.25%) compared with adipsin (Tab. 4).

Significant correlations of serum adipsin, osteopontin, and irisin levels with metabolic parameters were identified in the PCOS group (Tab. 5). HDL showed significant negative correlations with all three molecules (adipsin: $r = –0.213$, $p = 0.037$; osteopontin: $r = –0.245$, $p = 0.016$; irisin: $r = –0.328$, $p < 0.001$). HOMA-IR was positively correlated with osteopontin ($r = 0.226$, $p = 0.027$) and irisin ($r = 0.356$, $p < 0.001$). Other correlations are presented in Table 5.

The combination of adipsin and osteopontin demonstrated high efficacy in predicting PCOS (AUC = 0.817), significantly outperforming the adipsin and irisin model (AUC = 0.604, $p < 0.001$) with a negative IDI (–0.238) and continuous NRI (–0.875). The combination of osteopontin and irisin was also effective (AUC = 0.805), with a positive IDI (0.209) and NRI (0.688). The three-molecule combination was not significantly different from the adipsin and osteopontin combination ($p = 0.402$), although it exhibited a positive IDI (0.032) and NRI (0.346), indicating efficacy (Tab. 6, Fig. 4A–C).

DISCUSSION

This study showed that patients with PCOS had higher serum adipsin and lower osteopontin levels relative to healthy women. After adjustments for age and obesity, we found

that serum osteopontin levels significantly differed, and age exerted a strong effect. The predictive power of osteopontin exceeded that of adipsin; the combination of osteopontin and adipsin demonstrated the highest predictive power among all tested combinations. Lower osteopontin levels, either alone or in combination with higher adipsin levels, may serve as predictive markers for the development of PCOS.

The relationship between serum osteopontin levels and PCOS has been previously investigated. The absence of differences in osteopontin levels between non-obese patients with PCOS and healthy women in some studies suggested a positive association between osteopontin and PCOS [16]. However, these findings may have varied due to heterogeneity in study populations. Contrary to our findings, Saklamaz et al. reported significantly higher circulating osteopontin levels in women with PCOS than in age- and BMI-matched controls [18]. Another Turkish study observed similar osteopontin levels regardless of PCOS status and obesity [15]. Although significant differences in age and BMI were present between patients with PCOS and healthy controls, we detected significantly lower osteopontin levels in the PCOS group. After adjustments for age and obesity, we found that both PCOS status and age significantly influenced serum osteopontin levels. Although our categorization of patients with PCOS as obese and non-obese revealed a significant difference, whereby higher osteopontin levels were observed in obese patients with PCOS and healthy controls — consistent with findings from other studies [18, 20] — no differences in serum osteopontin levels were evident after adjustment for obesity. Wang et al. reported elevated circulating osteopontin levels in women with PCOS who displayed higher BMI, even if they were not classified as obese [16]. The greater proportion of non-obese patients with PCOS in our study may explain the contradictory findings regarding osteopontin levels: lower levels in the PCOS group relative to controls, but higher levels in obese patients with PCOS relative to non-obese patients. These results suggest that age, obesity, and PCOS status collectively have substantial effects on serum osteopontin levels. Further large-scale studies are required to clarify these relationships.

Previous studies have explored correlations of serum adipsin, osteopontin, and irisin levels with various demographic, clinical, and laboratory parameters that reflect the metabolic status of patients with PCOS [4, 18, 21]. Because osteopontin is a multifunctional proinflammatory cytokine, its levels may be associated with inflammation and insulin resistance in adipose tissues, contributing to insulin resistance [16, 18]. Although we observed significant correlations of BMI, waist circumference, fasting blood glucose, insulin, HOMA-IR, free testosterone, C-reactive protein, and triglycerides with circulating osteopontin levels

in patients with PCOS, these correlations were weak (for example coefficients were < 0.4). Similar correlations have been noted in healthy volunteers [18]. Comparable evaluations have been conducted in other studies for adipsin and irisin [15, 21, 22]. In the present study, we also identified similar weak but significant correlations of adipsin, osteopontin, and irisin levels with various laboratory parameters, including fasting blood glucose, insulin, HOMA-IR, HbA_{1c}, and HDL. Counter relationships between metabolic changes and serum levels of adipsin, osteopontin, and irisin may occur. Prospective cause-and-effect studies are required to elucidate the precise pathophysiological mechanisms and to determine whether these molecules contribute to the development of metabolic changes or are secreted as a consequence of such clinical conditions in patients with PCOS.

The impact of PCOS on serum irisin levels has been extensively investigated. Several studies have reported higher irisin levels in women with PCOS than in healthy controls [12, 21–24]. Elevated irisin levels have also been associated with hyperandrogenic PCOS [22]. Conversely, other studies have identified reduced irisin levels in obese patients with PCOS [14]. Notably, serum irisin levels were significantly lower in obese women with PCOS than in non-obese patients with PCOS [10, 14]. Behboudi-Gandevani et al. [7] reported no association between irisin levels and BMI in women with or without PCOS. Regarding the relationship between obesity and irisin levels, Chang et al. [23] observed higher serum irisin levels in overweight women (BMI > 25 kg/m^2), irrespective of PCOS status, and in overweight patients with PCOS from China. Similar findings have been reported by other authors [24]. Considering these findings, the brown adipose differentiation factor irisin is considered a potential biomarker for PCOS; it may increase diagnostic accuracy and help to mitigate the metabolic disorders associated with PCOS [8, 9, 21, 23].

Contrary to these observations, we detected similar irisin levels between the PCOS and control groups. However, a positive association between obesity and serum irisin levels was observed exclusively in patients with PCOS. Obese patients with PCOS had higher serum irisin levels relative to non-obese women with PCOS, which contrasts with findings by Wang et al. [14]. As noted in Lin's systematic review and meta-analysis [25], significant heterogeneity among studies—arising from differences in patient and PCOS-related features, as well as variations in diagnostic criteria—might contribute to such conflicting results. Further detailed investigations are necessary to conclusively address this issue.

Conflicting findings have also been reported regarding serum adipsin levels in patients with PCOS [1, 2, 4, 6]. Several studies have documented significantly elevated adipsin levels in women with PCOS and positive correlations with BMI, HOMA-IR, and free testosterone.

Obesity has also been significantly associated with higher circulating adipsin levels [4]. However, no significant differences in serum adipsin levels were observed between hyperandrogenic and normoandrogenic patients with PCOS and healthy controls [2].

Previous studies have evaluated the optimal cut-off values of various biomarkers, including irisin, with differing sensitivities and specificities [12, 21]. Luo et al. investigated the combined use of three cytokines (irisin, betatrophin, and Zinc-α2-glycoprotein) to diagnose PCOS. Their analysis showed that logistic regression incorporating the three circulating cytokines produced a higher AUC value, with improved sensitivity and specificity [21]. Our study analyzed the predictive power of various combinations of adipsin, osteopontin, and irisin. Based on the significant differences in serum adipsin and osteopontin levels between the PCOS and control groups, these two molecules were regarded as references for predicting PCOS. Other double and triple marker combinations demonstrated lower predictive power for PCOS diagnosis relative to the adipsin and osteopontin combination. Osteopontin, either alone or in combination with adipsin, may serve as a diagnostic marker for PCOS. Nevertheless, further studies are required to elucidate the clinical implications and explore the potential of these biomarkers in guiding therapeutic decisions.

To our knowledge, this study is the first to evaluate irisin, adipsin, and osteopontin levels together in patients with PCOS, which is a major strength of this research. The inclusion of healthy women as controls enhanced the accuracy of the findings. However, significant differences in age and BMI between the groups represent a major limitation. Future studies with age- and BMI-matched control groups are needed to produce more robust findings. The observational study design, which precludes causality analysis, is another limitation.

CONCLUSIONS

This study revealed that patients with PCOS had higher adipsin levels and lower osteopontin levels than healthy women, suggesting potential associations between these molecules and PCOS. However, after adjustments for age and obesity, the significant differences in osteopontin levels were influenced by age, highlighting the need for further research to clarify the relationship between osteopontin and PCOS. Irisin levels were similar between patients with PCOS and healthy controls, but obesity was linked to higher irisin levels in patients with PCOS. Osteopontin, either alone or in combination with adipsin, may represent a novel diagnostic biomarker for PCOS. These findings provide insights into the

potential roles of adipsin, osteopontin, and irisin in PCOS and its associated metabolic changes. However, further investigation is necessary to fully understand their mechanisms and clinical implications.

Article information and declarations

Ethical statement

The local ethical committee of Duzce University approved the study (Ethics Committee Reference Number: 2022/110). The researchers agreed to apply the principles of the Helsinki Declaration. All patients gave written informed consent. The ethics committee of Duzce University approved the study (reference no. 2022/110). The principles of the Declaration of Helsinki were followed. Informed consent was obtained from all participants.

Author contributions

FND, EY and BK developed project, analyzed and interpreted the patient data , and wrote the manuscript. AB was a major contributor in writing the manuscript. All authors read and approved the final manuscript. All authors approved the publication of this manuscript.

Data availability statement

The datasets used and analyzed during this study are available from the corresponding author upon reasonable request.

Funding

No funding was received for this study.

Acknowledgments

The authors thank all study participants.

Conflict of interest

All authors declare that they have no conflicts of interest.

REFERENCES

1. Martínez-García MÁ, Moncayo S, Insenser M, et al. Metabolic cytokines at fasting and during macronutrient challenges: influence of obesity, female androgen excess and sex. Nutrients. 2019; 11(11), doi: 10.3390/nu11112566, indexed in Pubmed: 31652917.
2. Daan NMP, Koster MPH, de Wilde MA, et al. Biomarker profiles in women with PCOS and PCOS offspring; a pilot study. PLoS One. 2016; 11(11): e0165033, doi: 10.1371/journal.pone.0165033, indexed in Pubmed: 27806063.
3. Butler AE, Moin AS, Sathyapalan T, et al. Components of the complement cascade differ in polycystic ovary syndrome. Int J Mol Sci. 2022; 23(20), doi: 10.3390/ijms232012232, indexed in Pubmed: 36293087.
4. Gursoy Calan O, Calan M, Yesil Senses P, et al. Increased adipsin is associated with carotid intima media thickness and metabolic disturbances in polycystic ovary syndrome. Clin Endocrinol (Oxf). 2016; 85(6): 910–917, doi: 10.1111/cen.13157, indexed in Pubmed: 27434652.
5. Kahyaoglu S, Ozaksit M, Duman GK, et al. Does baseline serum androgen levels have an impact on ovulation induction cycle outcomes by using clomifene citrate among infertile women with polycystic ovary syndrome? A retrospective cohort study. Gynecol Obstet Reproductive Med. 2023; 29(2): 122–127, doi: 10.21613/gorm.2022.1371.
6. Vejrazkova D, Lischkova O, Vankova M, et al. Distinct response of fat and gastrointestinal tissue to glucose in gestational diabetes mellitus and polycystic ovary syndrome. Physiol Res. 2017; 66(2): 283–292, doi: 10.33549/physiolres.933366, indexed in Pubmed: 27982680.
7. Behboudi-Gandevani S, Hedayati M, Mansournia M, et al. The associations between serum concentrations of irisin and glucose-dependent insulinotropic polypeptide with body mass index among women with and without polycystic ovary syndrome. Int J Endocrinol Metab. 2021; 19(4): e111914, doi: 10.5812/ijem.111914.
8. Weng Y, Zhang Y, Wang D, et al. Exercise-induced irisin improves follicular dysfunction by inhibiting IRE1α-TXNIP/ROS-NLRP3 pathway in PCOS. J Ovarian Res. 2023; 16(1): 151, doi: 10.1186/s13048-023-01242-x, indexed in Pubmed: 37525261.
9. Zheng Y, He J, Yang D, et al. Irisin reduces the abnormal reproductive and metabolic phenotypes of PCOS by regulating the activity of brown adipose tissue in mice. Biol Reprod. 2022; 107(4): 1046–1058, doi: 10.1093/biolre/ioac125.

10. Cao S, Hu Y. Effects of serum irisin, neuregulin 4, and weight management on obese adolescent girls with polycystic ovary syndrome. Biosci Rep. 2021; 41(9), doi: 10.1042/BSR20211658, indexed in Pubmed: 34427289.
11. Gür FM, Timurkaan S, Yalcin MH, et al. Immunohistochemical localization of irisin in mole rats (Spalax leucodon). Biotech Histochem. 2017; 92(4): 245–251, doi: 10.1080/10520295.2017.1303194, indexed in Pubmed: 28409694.
12. Onat T, Inandiklioglu N, Kara M, et al. Increased serum myonectin and irisin levels with myonectin and FNDC5 expressions in polycystic ovary syndrome: a case control study. J Obstet Gynaecol. 2022; 42(5): 1381–1387, doi: 10.1080/01443615.2021.1980516, indexed in Pubmed: 34907845.
13. Li M, Yang M, Zhou X, et al. Elevated Circulating Levels of Irisin and the Effect of Metformin Treatment in Women With Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2015; 100(4): 1485–1493, doi: 10.1210/jc.2014-2544.
14. Wang W, Guo Y, Zhang X, et al. Abnormal irisin level in serum and endometrium is associated with metabolic dysfunction in polycystic ovary syndrome patients. Clin Endocrinol (Oxf). 2018; 89(4): 474–480, doi: 10.1111/cen.13805, indexed in Pubmed: 29972694.
15. Alatas SE, Yavuz Dogu S, Kilic D, et al. Comparison of serum adiponectin and osteopontin levels along with metabolic risk factors between obese and lean women with and without PCOS. Endokrynol Pol. 2020; 71(6): 497–503, doi: 10.5603/EP.a2020.0074, indexed in Pubmed: 33125692.
16. Wang Y, Zhou W, Wu C, et al. Circulating osteopontin and its association with liver fat content in non-obese women with polycystic ovary syndrome: a case control study. Reprod Biol Endocrinol. 2018; 16(1), doi: 10.1186/s12958-018-0331-4.
17. Paravati R, Mello NDe, Onyido EK, et al. Differential regulation of osteopontin and CD44 correlates with infertility status in PCOS patients. J Mol Med. 2020; 98(12): 1713–1725, doi: 10.1007/s00109-020-01985-w.
18. Saklamaz A, Calan M, Yilmaz O, et al. Polycystic ovary syndrome is associated with increased osteopontin levels. Eur J Endocrinol. 2016; 174(4): 415–423, doi: 10.1530/EJE-15-1074, indexed in Pubmed: 26701868.
19. Kwon S, Heo A, Chun S. Triglyceride and glucose index for identifying abnormal insulin sensitivity in women with polycystic ovary syndrome. Obstet Gynecol Sci. 2023; 66(4): 307–315, doi: 10.5468/ogs.23103, indexed in Pubmed: 37376795.

20. Catalán V, Gómez-Ambrosi J, Rodríguez A, et al. Plasma osteopontin levels and expression in adipose tissue are increased in obesity. J Clin Endocrinol Metab. 2007; 92(9): 3719–3727, doi: 10.1210/jc.2007-0349, indexed in Pubmed: 17595250.
21. Luo P, Zhang C, He Y, et al. Several circulating biomarkers for PCOS diagnosis. Expe Clin Endocrinol Diabetes. 2019; 129(10): 705–712, doi: 10.1055/a-1025-3711.
22. Zhang L, Fang X, Li L, et al. The association between circulating irisin levels and different phenotypes of polycystic ovary syndrome. J Endocrinol Invest. 2018; 41(12): 1401–1407, doi: 10.1007/s40618-018-0902-4, indexed in Pubmed: 29785700.
23. Chang CL, Huang SYu, Hsu YaC, et al. The serum level of irisin, but not asprosin, is abnormal in polycystic ovary syndrome patients. Sci Rep. 2019; 9(1): 6447, doi: 10.1038/s41598-019-42061-9, indexed in Pubmed: 31015585.
24. Farhan FS, Hussien SS. Irisin as a novel marker for insulin resistance in iraqi women with polycystic ovary syndrome before and after metformin therapy. J Obstet Gynaecol India. 2019; 69(Suppl 2): 194–200, doi: 10.1007/s13224-018-1176-7, indexed in Pubmed: 31686756.
25. Lin K, Sun X, Wang X, et al. Circulating adipokine levels in nonobese women with polycystic ovary syndrome and in nonobese control women: a systematic review and meta-analysis. Front Endocrinol (Lausanne). 2021; 11, doi: 10.3389/fendo.2020.537809.

Table 1. Sociodemographic and clinical characteristics of the study groups

	Groups		p
	PCOS (n = 96)	**Control (n = 80)**	
Age (year)§	22.0 [18.0–40.0]	29.0 [19.0–48.0]	< **0.001****
Educational status‡			
Primary	4 (4.2)	8 (10.0)	0.077*
High school/college	30 (31.2)	15 (18.8)	
University	62 (64.6)	57 (71.2)	
Smoking‡	15 (15.6)	25 (31.2)	**0.022***
Alcohol‡	10 (10.4)	13 (16.2)	0.358*
Height (cm)§	163.0 [145.0–175.0]	163.0 [149.0–179.0]	0.769**
Weight (kg)§	62.5 [43.0–115.0]	60.0 [39.0–97.0]	0.069**
BMI (kg/m^2)§	23.6 [17.3–40.0]	22.1 [15.2–37.0]	**0.039****
Obesity (BMI ≥ 30 kg/m^2)‡	23 (24.0)	7 (8.8)	**0.013***
Waist to hip ratio§	0.8 [0.7–1.0]	0.8 [0.6–1.3]	0.119**
Modified Ferriman–Galleway score§	11.0 [0.0–24.0]	3.0 [0.0–7.0]	< **0.001****

Hirsutism (≥ 8) ‡	75 (78.1)	0 (0.0)	**< 0.001***

§ median [min–max]; ‡ n (%); * Pearson Chi-Square or Fisher Freeman Halton test; ** Mann–Whitney U test; BMI: body mass index; PCOS — polycystic ovary syndrome

Table 2. Comparison of the groups according to the laboratory investigations

	Groups		**p**
	PCOS (n = 96)	**Control (n = 80)**	
Fasting blood glucose (mg/dL) §	90.6 [68.1–121.3]	91.8 [56.8–129.4]	0.162*
Fasting insulin (mIU/L) §	10.3 [0.4–73.4]	8.5 [1.7–51.3]	**0.008***
HOMA-IR §	2.4 [0.1–15.2]	1.9 [0.4–11.8]	**0.030***
HbA_{1c} §	5.0 [4.5–5.8]	5.0 [4.2–8.0]	0.600*
Cholesterol (mg/dL) †	167.9 ± 36.0	161.9 ± 25.0	0.193**
LDL (mg/dL) §	88.5 [33.3–184.5]	88.8 [39.4–151.1]	0.747*
HDL (mg/dL) §	50.5 [26.9–97.8]	54.0 [28.6–90.4]	0.098*
Triglyceride (mg/dL) §	91.4 [39.7–329.2]	72.4 [30.5–231.8]	**0.002***
Triglyceride/glucose index §	4.5 [3.0–5.2]	4.4 [4.0–5.0]	**0.011***
FSH (mIU/mL) §	5.6 [2.5–9.3]	6.7 [3.1–18.6]	**< 0.001***
LH (mIU/mL) §	7.8 [1.3–25.8]	6.2 [1.5–16.1]	**0.003***
LH/FSH §	1.4 [0.2–4.5]	0.9 [0.3–3.4]	**< 0.001***
E2 (pg/ml) §	37.9 [8.6–143.1]	44.8 [5.0–292.4]	0.071*
Total testosterone (nmol/L) †	0.4 ± 0.2	0.3 ± 0.1	**< 0.001****
Prolactin (mIU/L) §	19.9 [6.8–72.0]	16.7 [8.2–52.9]	0.076*
TSH (uIU/mL) §	2.0 [0.4–11.2]	1.9 [0.0–7.0]	0.537*
CRP (mg/dL) §	0.1 [0.1–3.4]	0.2 [0.1–4.2]	0.287*
Adipsin (ng/mL) §	18.9 [5.0–45.2]	16.0 [5.8–40.4]	**0.020***
Osteopontin (ng/mL) §	1.6 [0.2–8.4]	3.1 [1.2–7.6]	**< 0.001***
Irisin (ng/mL) §	247.3 [10.6–1152.2]	244.7 [25.9–1200.0]	0.747*

§ median [min–max]; † mean ± standard deviation; ‡ n (%); * Mann–Whitney U test; ** Independent Samples T-Test; CRP — C-reactive protein; E2 — estradiol; FSH — follicle-stimulating hormone; HbA_{1c} — glycated hemoglobin; HDL — high-density lipoprotein; HOMA-IR — homeostasis assessment insulin resistance index; LDL — low-density lipoprotein; LH — luteinizing hormone; PCOS — polycystic ovary syndrome; TSH — thyroid stimulating hormone

Table 3. Adipsin, osteopontin, and irisin levels in obese and non-obese polycystic ovary syndrome patients and healthy controls

	PCOS		p*	Control		p*
	BMI < 30 kg/m² (n = 73)	**BMI ≥ 30 kg/m² (n = 23)**		**BMI < 30 kg/m² (n = 73)**	**BMI ≥ 30 kg/m² (n = 7)**	
Adipsin (ng/mL)§	18.3 [5.0–45.2]	20.4 [8.4–41.6]	0.105	15.7 [5.8–40.4]	20.4 [13.2–26.3]	0.336
Osteopontin (ng/mL)§	1.4 [0.2–8.4]	1.9 [0.4–6.7]	**0.040**	3.2 [1.2–7.6]	2.9 [1.4–5.1]	0.598
Irisin (ng/mL)§	164.5 [10.6–1152.2]	468.5 [73.1–884.5]	**< 0.001**	209.9 [25.9–1200.0]	393.5 [98.5–939.4]	0.068

§ median [min–max]; * Mann–Whitney U test; BMI — body mass index; PCOS — polycystic ovary syndrome

Table 4. The receiver operating characteristics curve analysis in predicting the development of polycystic ovary syndrome for adipsin, osteopontin, and irisin

	AUC	Sensitivity	Specificity	Cut-off	95 % CI	p
Adipsin (ng/mL)	0.602	69.79	51.25	> 16.03	0.526–0.675	**0.017**
Osteopontin (ng/mL)	0.802	71.87	76.25	≤ 2.27	0.735–0.858	**< 0.001**
Irisin (ng/mL)	0.514	25.00	85.00	≤ 90.64	0.438–0.590	0.745

AUC — area under the curve; CI — confidence interval; PCOS — polycystic ovary syndrome

Table 5. Correlation analysis of serum adipsin, osteopontin, and irisin levels with laboratory parameters related to the metabolic status of polycystic ovary syndrome

		Adipsin	Osteopontin	Irisin
Fasting blood glucose	r	–0.278	0.192	0.157
	p	**0.006**	0.061	0.127
Fasting insulin	r	0.169	0.220	0.371
	p	0.100	**0.031**	**< 0.001**
HOMA-IR	r	0.124	0.226	0.356
	p	0.230	**0.027**	**< 0.001**
HbA_{1c}	r	–0.339	–0.066	0.252
	p	**< 0.001**	0.525	**0.013**
Cholesterol	r	0.009	0.038	0.001
	p	0.929	0.711	0.994
LDL	r	–0.044	0.097	0.106

	p	0.673	0.346	0.304
HDL	R	–0.213	–0.245	–0.328
	P	**0.037**	**0.016**	**0.001**
Triglyceride	r	0.192	0.172	0.093
	p	0.060	0.094	0.367
Total testosterone	r	0.056	0.188	0.164
	p	0.588	0.066	0.110

Spearman's rho correlation coefficient was used.
HbA_{1c} — glycated hemoglobin; HDL — high-density lipoprotein; HOMA-IR — homeostasis assessment insulin resistance index; LDL — low-density lipoprotein

Table 6. Comparative evaluation of predictive models for polycystic ovary syndrome using AUC, IDI, and NRI metrics

Prediction model	AUC	95 % CI	p value for AUC difference	IDI	95 % CI	p value for IDI	NRI	95 % CI	p value for NRI
Adipsin + osteopontin (reference)	0.817	0.755–0.88							
Adipsin + irisin	0.604	0.521–0.688	**< 0.001**	–0.238	–0.299–0.177	**< 0.001**	–0.875	–1.142–0.608	**< 0.001**
Osteopontin + irisin	0.805	0.742–0.868	**< 0.001**	0.209	0.14–0.278	**< 0.001**	0.688	0.409–0.966	**< 0.001**
Adipsin + osteopontin + irisin	0.816	0.753–0.879	0.402	0.032	0.006–0.057	**0.014**	0.346	0.054–0.638	**0.020**

AUC — area under the receiver operating characteristic curve; CI — confidence interval; IDI — integrated discrimination improvement; NRI — net reclassification improvement

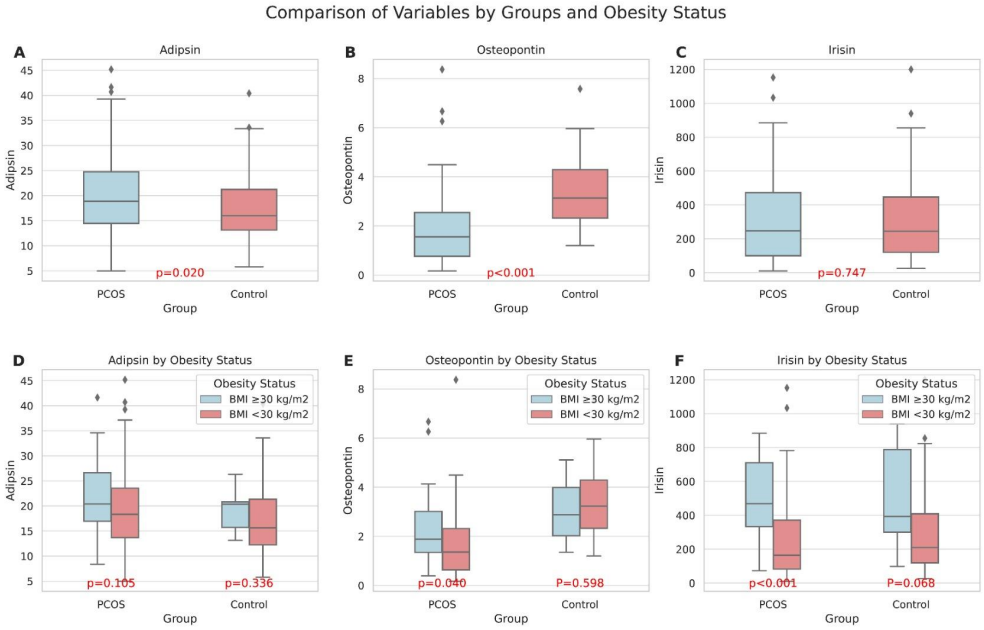

Figure 1. Serum levels of adipsin, osteopontin, and irisin in polycystic ovary syndrome and control groups; **A–C** levels without differentiation by obesity status; **D–F** levels categorized by obesity status (body mass index ≥ 30 kg/m^2)

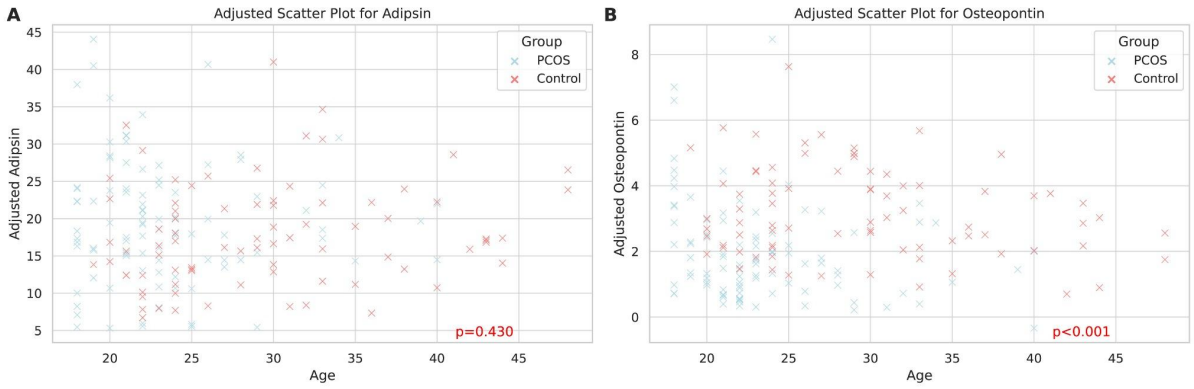

Figure 2. Scatter plot matrix of adipsin and osteopontin in relation to age; **A.** relationship between adipsin and age; **B.** correlation of osteopontin with age

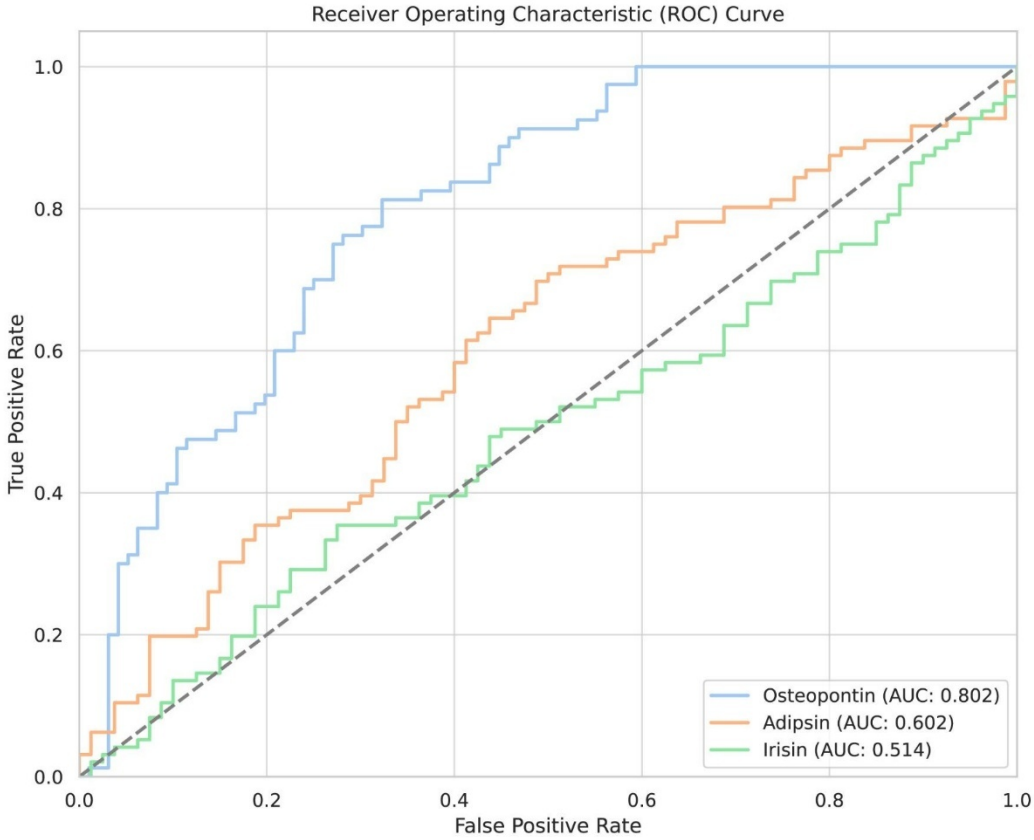

Figure 3. Receiver operating characteristic curve analysis for predicting polycystic ovary syndrome based on different biomarkers: circulating adipsin, osteopontin, and irisin

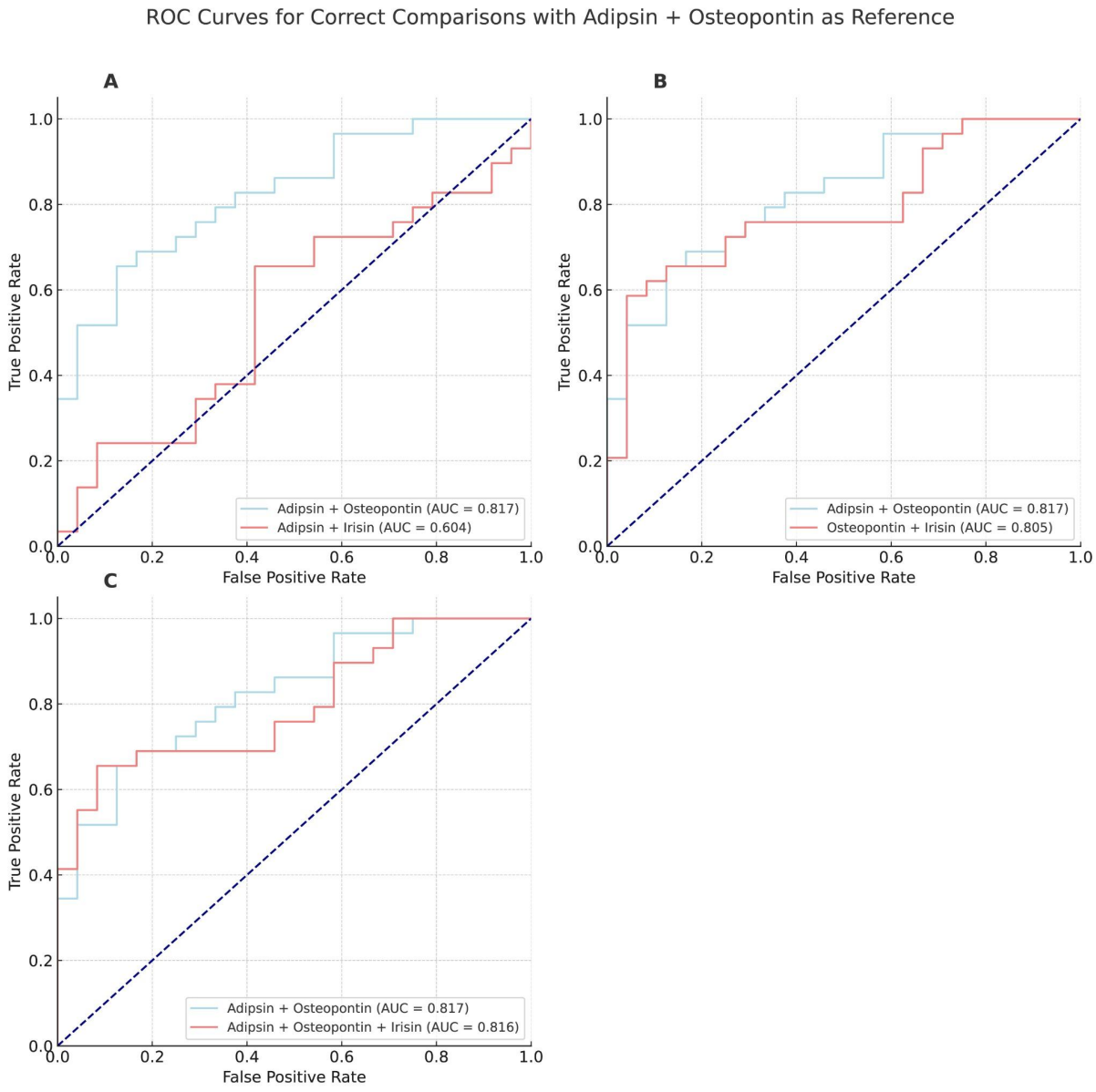

Figure 4. Receiver operating characteristic curve analysis for predicting polycystic ovary syndrome; comparing combinations of circulating adipsin, osteopontin, and irisin with 'adipsin + osteopontin' as the reference; **A.** adipsin + irisin; **B.** osteopontin +irisin; **C.** adipsin + osteopontin + irisin