Exploring some pro-inflammatory cytokines and adipokines as novel biomarkers in polycystic ovary syndrome

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ABSTRACT

Non-communicable diseases are more likely to affect women with polycystic ovarian syndrome (PCOS), in particular, mostly ascribed to the existence of low-grade chronic inflammation brought on by adipokines and proinflammatory cytokines. Angiopoietin-2, lipocalin-2, adipsin, and interleukin (IL)-22 concentrations in women with PCOS were assessed in this study, and their levels were compared to those in a healthy control group. Blood samples were obtained from outpatient and private gynecological clinics, as well as primary healthcare settings, from 55 women diagnosed with PCOS and 35 healthy control women. The serum concentrations of proinflammatory cytokines and adipokines were evaluated using the enzyme-linked immunosorbent assay technique. The data revealed that serum concentrations of angiopoietin-2, lipocalin-2, adipsin, and IL-22 were markedly increased in the PCOS cohort relative to the control cohort. Lipocalin-2 revealed a substantial positive correlation with adipsin, angiopoietin-2, and IL-22; likewise, angiopoietin-2 revealed a large positive correlation with adipsin and IL-22. Receiver operating characteristic curve analysis indicated that both lipocalin-2 and adipsin exhibited enhanced diagnostic efficacy for PCOS [area under the curve (AUC)=0.8518; AUC=0.8103, respectively], while angiopoietin-2 and IL-22 demonstrated moderate diagnostic significance for PCOS (AUC=0.7476; AUC=0.7340, respectively). Proinflammatory cytokines and adipokines showed significant diagnostic potential for identifying PCOS and may serve as markers for screening, thereby enhancing diagnostic accuracy for PCOS. These results suggest that lipocalin-2, adipsin, angiopoietin-2, and IL-22 could be utilized as biomarkers for assessing the risk of PCOS.

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Key words: polycystic ovary syndrome, pro-inflammatory cytokines, adipokines.

Contributions: both authors equally contributed to manuscript preparation.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval and consent to participate: ethical approval was obtained from Salahaddin University Ethics Committee [Approval No. 18209 (2024) and 13796].

Informed consent: participants signed an informed consent form after being informed that participation in the study was entirely voluntary for all patients.

Patient consent for publication: all patients were informed about the study's objectives, procedures, potential risks, and benefits, and they voluntarily agreed to participate by providing written informed consent

Availability of data and materials: all data generated during this study are included in this published article

Funding: none.

Acknowledgments: the authors would like to thank Salaheddin University for their support.

Received: 18 April 2025.
Accepted: 17 June 2025.
Early view: 11 September 2025.

Licensee PAGEPress, Italy
Italian Journal of Medicine 2025; 19:2023
doi:10.4081/itjm.2025.2023

Introduction

One of the main causes of infertility in women of reproductive age is polycystic ovarian syndrome (PCOS), a common gynecological condition characterized by a variety of endocrine symptoms,[1,2] after non-classical congenital adrenal hyperplasia and hyperprolactinemia were rejected as specific alternative diagnoses.[3] PCOS is a condition marked by elevated androgen levels (exemplified by hirsutism and/or hyperandrogenemia) and ovarian dysfunction symptoms (such as oligo-ovulation and/or polycystic ovarian morphology). Compared to other women in the reproductive age group, premenopausal women have a higher prevalence of PCOS, which ranges from 6% (based on outdated, more restrictive criteria) to 20% (based on more recent, broader definitions).[4] After ruling out other possible causes for elevated androgen levels, the National Institutes of Health identified PCOS in 1990 by observing monthly irregularities and clinical or biochemical markers of hyperandrogenism.

Pro-inflammatory cytokines contribute to the activation and recruitment of immune cells, and anti-inflammatory cytokines help to suppress excessive inflammation.

The origin of PCOS could exert influence on the inconsistency between pro-inflammatory cytokines that contribute to the activation and recruitment of immune cells, and anti-inflammatory cytokines, which help to suppress excessive inflammation.[5] Thus, inflammatory reactions serve as mediators and exacerbate the metabolic characteristics of PCOS. Obesity is probably linked to both PCOS and insulin resistance, as adipose cells play a vital role in the production of pro-inflammatory substances, causing persistent inflammation.[6]

Adipose tissue is the primary source of cytokine synthesis, which causes the organism to go into a pro-inflammatory state.[7] There is a lack of understanding regarding the specific mechanisms underlying inflammation in women with PCOS. An increase in adipocytes and immune cell infiltration within adipose tissue is a sign of excess adiposity, which is a notable prevalence of PCOS. As a result, low-grade inflammation persists.[8]

Adipose tissue operates as a dynamic organ that releases cytokines, hormones, adipokines, and influences the endocrine systems that regulate immunity, inflammation, metabolic pathways of glucose and lipid, as well as reproductive functions.[9] Excess visceral fat in females with PCOS may trigger the persistent presence of elevated levels of circulating cytokines mediated by pro-inflammatory cytokines.[10]

Angiogenesis plays a vital role in the progression of ovarian follicles and the ovulation mechanism.[11] Angiogenesis is governed by several proteins and growth factors, including angiopoietins. Angiopoietin-2 acts as an antagonist to angiopoietin-1 and is crucial in the control of angiogenesis processes.[12] Angiopoietin-2 is involved in follicle formation, showing elevated levels during follicular expansion and reduced levels during follicle maturation.[13,14] Research employing animal models of PCOS has shown reduced levels of angiopoietin-2, leading to increased ovarian vascularity and vessel stability.[15] Altered angiopoietin-2 expression was associated with a deficiency in oocyte maturation.[16] A prior study has recorded increased levels of angiopoietin-2 in follicular fluid among PCOS subjects undergoing ovarian stimulation.[17]

The respiratory and gastrointestinal systems, the genitourinary system, endothelial cells, vascular smooth muscle cells, hepatocytes, endometrial cells, and splenic cells are among the tissues that have been shown to express the adipocytokine lipocalin-2.[18] Numerous studies have reported varying levels of lipocalin in both PCOS and other illnesses interrelated with insulin resistance, indicating increases, decreases, or no changes; however, the underlying mechanisms contributing to these inconsistent findings remain incompletely understood.[19-21] Since its expression increases with agents that cause insulin resistance, lipocalin-2 seems to facilitate the development of insulin resistance, whereas lowering its expression in cultured adipocytes improves insulin efficacy.[22] Two of the main characteristics of PCOS are obesity and insulin resistance. On the other hand, little is known about the levels of serum lipocalin-2 in PCOS patients.[23] The study's objective was to assess the levels of adipokines, chemokines, and proinflammatory mediators in the serum of patients with PCOS. This research intends to examine the association between changes in proinflammatory mediators and adipokines in PCOS patients.

Materials and Methods

Study design

The study involved 55 participants with a diagnosis of PCOS (aged 15-48) and 35 healthy women (aged 15-48) who visited our Gynecology and Obstetrics Clinic. The identification of PCOS was substantiated following globally accepted evidence-based protocols, specifically the Rotterdam indicator, which requires the existence of a minimum of two of the following signs: ovarian dysfunction (oligo-amenorrhea), biochemical and/or clinical high levels of androgens, and enlarged polycystic ovaries. The experimental group comprised females exhibiting normal menstrual cycles, absence of ovulatory abnormalities, standard basal hormone levels, and polycystic ovarian morphology in both ovaries. Criteria for exclusion included: i) endocrine disorders impacting reproductive function; ii) other conditions causing hyperandrogenemia and ovulation dysfunction; iii) the usage of hormone treatment within the past 3 months; iv) pregnancy, hyperprolactinemia, breastfeeding, Cushing syndrome, congenital adrenal hyperplasia, alternative adrenal gland disorders, thyroid disorders, and women utilizing hormonal contraceptive methods.

Sample collection

In the first follicular phase (days 2-5) of the menstrual cycle, 5 mL of venous blood was extracted from the antecubital vein by peripheral venipuncture. The specimens were gathered in uncoated tubes devoid of anticoagulants and subsequently processed *via* centrifugation. The resultant sera were aliquoted into three Eppendorf tubes to avoid repetitive freeze-thaw cycles and subsequently stored at low temperatures until analysis. Serum concentrations of angiopoietin-2, lipocalin-2, adipsin, and interleukin (IL)-22 were quantified for both the patient and control cohorts *via* the enzyme-linked immunosorbent assay technique.

Statistics

In the data processing for this research, both descriptive and inferential statistical methods were employed using the GraphPad Prism software. The data evaluation was conducted at a 95% confidence interval, with results presented as mean ± standard error. Given that many of the studied parameters exhibited a normal Gaussian distribution, the parametric *t*-test method was applied. Correlation analysis among the parameters was performed using Pearson's correlation coefficient. The diagnostic effectiveness of PCOS patients was assessed using receiver operating characteristic (ROC) curves, as well as the ideal threshold value, area under the curve (AUC), sensitivity, and specificity for each index. P was taken into consideration as the significance level.

Results

Serum levels of adipokines in polycystic ovarian syndrome

The concentrations of lipocalin and adipsin in both cohorts, along with the statistically meaningful differences observed in these adipokines, are presented in *Supplementary Table 1* and Figures 1 and 2.

Circulating concentration of adipsin in polycystic ovarian syndrome

The mean serum levels of adipsin in PCOS patients were elevated (294.0±149.8 pg/mL) compared to control women (144.2±73.46 pg/mL), as shown in *Supplementary Table 1* and Figure 2.

Serum levels of proinflammatory factors

Supplementary Table 2 illustrates a remarkable difference in the concentrations of proinflammatory factors between the two study groups.

Circulating concentration of angiopoietin-2 in polycystic ovarian syndrome

Data presented in *Supplementary Table 2* and Figure 3 demonstrate that the mean circulating concentration of angiopoietin-2 in PCOS patients was remarkably elevated (24.23±12.37 ng/mL) compared to that in control women (11.86±6.174 ng/mL).

Circulating concentration of interleukin-22 in polycystic ovarian syndrome

Data in *Supplementary Table 2* and Figure 4 demonstrate that the mean circulating concentration of IL-22 in PCOS patients was higher (17.16±7.752 pg/mL) compared to those in control women (9.407±3.739 pg/mL).

The receiver operating characteristic curve analysis

The ROC curve analysis was conducted to assess the factors influencing the dependent variables for diagnosing PCOS. The sensitivity of lipocalin-2, adipsin, angiopoietin-2, and IL-22 as biomarkers for PCOS risk was 78%, 81%, 72%, and 77%, respectively, with specificities of 94%, 97%, 91%, and 84%, and 100%, respectively (Table 1, *Supplementary Figures 1-4*).

Table 1 illustrates the AUC, specificity, and sensitivity of various proinflammatory factors and adipokines in patients with PCOS when compared to control groups. An AUC ranging from 0.9 to 1.0 indicates outstanding predictive ability

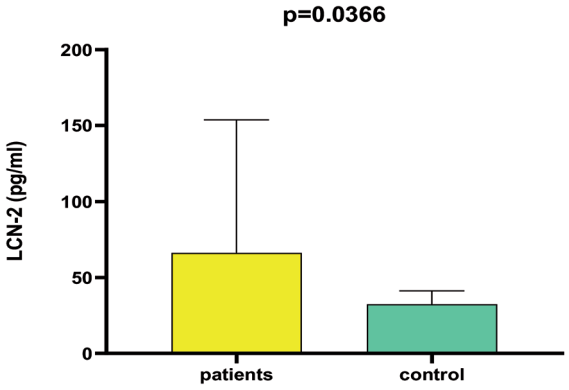

Figure 1. Serum lipocalin-2 concentration comparison between the patient and control groups.

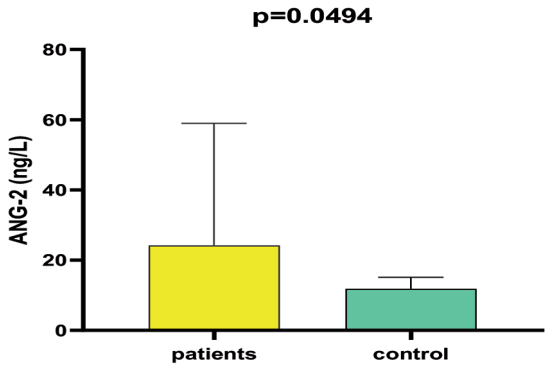

Figure 3. Serum angiopoietin-2 concentration comparison between the patient and control groups.

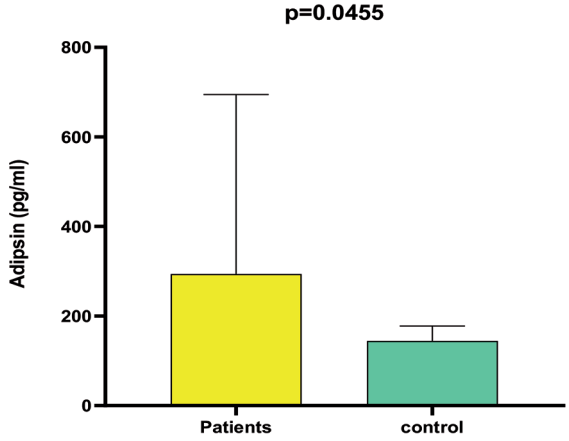

Figure 2. Serum adipsin concentration comparison between the patient and control groups.

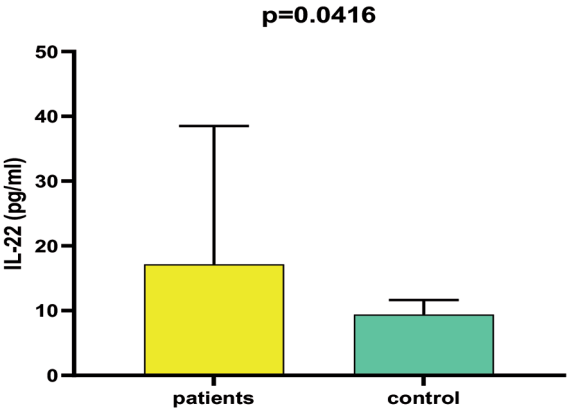

Figure 4. Serum interleukin-22 concentration comparison between the patient and control groups.

for a biomarker, while a range of 0.8 to 0.9 suggests a highly effective marker. An AUC between 0.6 and 0.7 reflects a satisfactory marker, and an AUC of 0.6 denotes an inconsequential marker. The diagnostic potential of lipocalin-2 was represented by an AUC of 0.8103, showing a sensitivity of 78 and a specificity of 94 (*Supplementary Figure 1*). The diagnostic ability of adipisin was measured with an AUC of 0.8518, demonstrating a sensitivity of 71 and a specificity of 97 (*Supplementary Figure 2*). The diagnostic ability of angiopoietin-2 was characterized by an AUC of 0.7476, exhibiting a sensitivity of 72 and a specificity of 91(*Supplementary Figure 3*). The diagnostic capability of IL-22 was measured with an AUC of 0.7340, demonstrating a sensitivity of 77 and a specificity of 84 (*Supplementary Figure 4*).

Relationship among parameters under study

Supplementary Figures 5-9 show the results of Pearson's correlation analysis. There was a significant positive correlation between lipocalin-2 and adipsin (r=0.8469; p<0.0001) as depicted in *Supplementary Figure 5*. Serum lipocalin-2 levels and IL-22 were found to be strongly positively correlated (r=0.5953; p<0.0001), as shown in *Supplementary Figure 6*. Lipocalin-2 demonstrated a remarkable positive correlation with angiopoietin-2 (r=0.7975; p<0.0001), as shown in *Supplementary Figure 7*. Serum angiopoietin-2 levels and IL-22 were found to be significantly positively correlated. (r=0.5390; p<0.0001) as shown in *Supplementary Figure 8*. A remarkable positive correlation exists between circulating angiopoietin-2 levels and adipsin (r=0.8201; p<0.0001) as illustrated in *Supplementary Figure 9*.

Discussion

Our study demonstrated a substantial elevation in serum lipocalin concentrations in patients with PCOS, in contrast to healthy control subjects, with a mean serum lipocalin concentration in the PCOS cohort of 66.12±33.72 pg/mL, in contrast to 32.40±15 pg/mL in the control group. The findings align with those of Cakal *et al*., who noted significantly increased serum lipocalin-2 concentration in patients with PCOS relative to controls matched for age and body mass index (BMI). On the other hand, several studies have shown that there are no appreciable differences in lipocalin-2 levels between PCOS patients and healthy controls.[19,24]

According to research, lipocalin-2 may be a biomarker for insulin resistance in PCOS patients. Our research showed that women with PCOS had higher lipocalin-2 concentrations.[20]

Several studies have suggested that lipocalin-2 and androgens may interact.[25-27] Rat studies have demonstrated the impact of androgens on the synthesis of lipocalin-2 and the presence of putative androgen response elements within the promoter regions of the genes encoding lipocalins.[27] Additionally, lipocalin-2 alters the activity of the enzyme aromatase, which is essential for the conversion of androgens into estrogens in granulosa cells and adipose tissue.[25,26] Garcia Martinez *et al.* demonstrated how women with PCOS had significantly higher levels of lipocalin-2 in their adipose tissue compared to their peers with the same BMI, but no such elevation was found in their skeletal muscle. Male participants were included in this study, indicating that lipocalin-2 production in PCOS-afflicted women exhibited characteristics more commonly found in men. The authors hypothesized that because the synthesis of many adipocytokines in women with complex PCOS reflects male-pattern characteristics, androgen concentrations may regulate the differences in the hormonal roles of adipose tissue between sexes.[26] The primary adipokine produced by adipose tissue is lipocalin-2. PCOS is a common endocrine condition affecting 6-18% of females during their reproductive age. This disorder is frequently associated with obesity, insulin resistance, an increased incidence of type II diabetes, and hyperlipidemia. The precise mechanisms underlying PCOS remain incompletely elucidated.[28]

Gencer *et al.* (2014) reported that lipocalin-2 concentrations were remarkably diminished in females with PCOS.[20,29] A remarkable decline in plasma lipocalin-2 concentrations was noted in females diagnosed with PCOS; this observation was not linked to elements such as insulin resistance, obesity, or estrogen concentrations. In contrast, it was demonstrated that lipocalin-2 levels were heightened in women with PCOS compared to the control cohort. Additionally, elevated lipocalin-2 levels were seen in women with PCOS. An examination of the literature reveals discrepancies among these studies, which are believed to arise from variations in the sample group characteristics examined.[30]

PCOS is reported to affect at least 1 in every 200 adolescent girls. Although the precise causes of PCOS development during adolescence remain unclear, certain allelic variants of genes and ecological factors that may be involved in predisposition have been identified.[31] PCOS in females is believed to exhibit polygenic inheritance influenced by environmental factors. Additionally, elevated inflammatory markers associated with PCOS indicate that inflammation may play a role, though its direct involvement in the pathogenesis is uncertain.[32] A significant association has also been noted between obesity and the onset of PCOS in adolescent girls.[31] Diagnosing PCOS in adolescents poses challenges and is contentious compared to adult women, relying on two clinical criteria: hyperandrogenism (either clinical or biochemical) and menstrual irregularity. Adolescents exhibiting only one of these characteristics may be deemed "at risk" for PCOS.[33] In recent

Table 1. The receiver operating characteristic curve analysis.

Variables of the ROC curve	AUC	95% CI	SE	p	Sensitivity (%)	Specificity (%)
Lipocalin	0.8103	0.7088-0.9117	0.05177	<0.0001	78	94
Adpisin	0.8518	0.7621-0.9414	0.04573	<0.0001	81	97
Angiopoietin-2	0.7476	0.6253-0.8700	0.06243	0.0006	72	91
IL-22	0.7340	0.6221-0.8459	0.05708	0.0005	77	84

ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence intevarl; SE, standard error.

decades, significant augmentations in the incidence the prevalence of overweight and obesity among females have occurred alongside expedited maturation and a pronounced rise in the cases of adolescent PCOS.[34]

Adolescents diagnosed with PCOS typically demonstrate hyperandrogenism and insulin resistance. The principal diagnostic criteria for adolescent PCOS include hirsutism, acne, seborrhea, androgen excess in females, and oligo-amenorrhea occurring more than 2 years post-menarche.[34,35]

Adipsin, referred to as complement factor D, is an enzyme with a cytokine configuration that links the metabolism of adipose tissue to complement system pathways. The association of adipsin concentrations with age, BMI, fasting plasma glucose, and leptin highlights its significance in the development of obesity.[36] Originally described as complement factor D, adipsin additionally activates the cascade reaction of the complement system, making it a crucial element of the immune system.[37] The complement system is one element that makes up the innate immune system. It is linked to insulin resistance, cardiovascular diseases, and inflammation.[38] Evidence indicates a correlation between PCOS and the complement system, suggesting that adipsin may potentially affect the condition. In our investigation, adipsin levels were markedly elevated in adolescent girls with PCOS relative to controls in good health. Nonetheless, although adipsin is a protein released by adipocytes, its concentrations were decreased in obese teenagers with PCOS compared to their average weight peers.

The present investigation reveals that circulating adipsin concentrations are markedly increased in people with PCOS relative to the control group cohort. Adipose tissue impairment occurs in women diagnosed with PCOS,[39,40] promoting the advancement of reproductive and metabolic disorders in these individuals. Adipsin, the primary protein produced by mature adipocytes, has a structural configuration similar to complement factor D, which functions as an enzyme that limits the rate at which the alternative complement mechanism is activated. This signifies a correlation between obesity and adipsian.[41] While levels of adipsin have been demonstrated to diminish in animal models of obesity, various human investigations suggest that this adipokine is increased in disorders of metabolism associated with obesity.[42,43] The cause of this difference is indeterminate. A study found higher adipsin levels in both cord blood and maternal blood of obese pregnant women compared to their slim counterparts. The research demonstrated a significant positive relationship between circulating adipsin level and BMI among pregnant individuals, in addition to a link between fetal adipsin levels and fetal birth weight.[44] Furthermore, an additional investigation indicated that the levels of circulating adipsin were elevated in individuals with obesity as opposed to those without. A notable proportion of women diagnosed with PCOS exhibit diverse degrees of insulin resistance.[40,45]

Adipose tissue functions as a primary target organ in people with PCOS. Those affected by PCOS demonstrate alterations in the synthesis and release of adipokines in both peripheral and visceral adipose tissue. Adipsin is an essential adipokine synthesized by adipose tissue.

The pathogenesis of PCOS is intricate. Some researchers propose that a chronic inflammatory response is the primary mechanism underlying the onset and progression of PCOS.[32] Numerous studies demonstrate that inflammatory indicators, including C-reactive protein and leukocyte count, are significantly heightened in individuals diagnosed with PCOS.[46] While clomiphene, an estrogen antagonist, is the traditional medication for infertility associated with PCOS, some patients do not respond to it. Given the significant role, this study intends to examine the notion that serum concentrations of cytokines that promote inflammation are linked to the pathophysiological processes of PCOS.

Angiogenesis is essential for the progression of ovarian follicles and the ovulation process.[11] It is regulated by several proteins and growth factors, including angiopoietins. Angiopoietin-2 acts as an antagonist to angiopoietin-1 and is crucial for the regulation of angiogenesis.[12] Angiopoietin-2 concentrations rise during follicular expansion and decline throughout follicular maturation.[13,14] Research on animal models of PCOS suggests that diminished angiopoietin-2 levels correlate with increased ovarian vascularity and vessel stability.[15] Abnormal angiopoietin-2 expression has been linked to issues in oocyte maturation.[16] A previous study found elevated angiopoietin-2 levels in the follicular fluid in females with PCOS receiving ovarian stimulation.

Proinflammatory factor angiopoietin-2 plays a vital function in the modulation of tissue and organ angiogenesis.[12] Under normal physiological conditions, angiopoietin-2 levels gradually increase with follicle development. As follicles mature, the levels of angiopoietin-2 subsequently decline, indicating its essential function in follicle development.[14] Prior animal models and human research have shown that angiopoietin-2 levels are markedly reduced in people with PCOS, resulting in a considerable increase in ovarian vascular density.[15,17] This study found that angiopoietin-2 levels in PCOS patients were considerably elevated compared to healthy women. Rajendiran *et al.* indicated that serum angiopoietin-2 levels in PCOS patients serve as a distinct risk factor for diseases.[47]

Prolonged inflammation, as demonstrated by changes in proinflammatory and anti-inflammatory cytokines, is a characteristic of PCOS. Cytokines play a vital role in the regulation of normal ovarian and menstrual cycles. The alteration of cytokines, a significant component in the chronic inflammatory mechanism related to PCOS, is believed to be associated with the diminished ovarian response to CC treatment.[48]

Meta-inflammation in PCOS is marked by elevated levels of various indicators, including inflammatory cytokines like IL-6 and IL-18. The immune response is influenced by the ratio of estrogen to progesterone.[49,50]

Patients with a diagnosis of PCOS exhibit reduced progesterone levels as a consequence of either oligo-ovulation or anovulation; consequently, heightened estrogen levels may result in an overactive immune response, ultimately resulting in the generation of autoantibodies in patients with PCOS.[51] Estrogen affects the immune system by encouraging Th2 lymphocytes, monocytes, T-lymphocytes, and Th1 cells to produce IL-4, IL-1, and IL-6, respectively. The immune response's activating effects of estrogens may be lessened by progesterone.

The systemic inflammatory state associated with PCOS is significantly influenced by localized ovarian inflammation. Granulosa cells isolated from PCOS patients showed markedly lower levels of inflammatory cytokines and associated gene expressions after receiving IL-22.[52] Qi *et al*. suggested that IL-22 may enhance adenosine monophosphate kinase and reduce the inflammatory state of macrophages by activating signal transducer and activator of transcription.

Mechanistic insight into the treatment of ovarian dysfunction is provided by the change in local inflammation brought on by IL-22.

IL-22, a constituent of the IL-10 family, has been thoroughly documented for its involvement in mitigating metabolic disorders. It has been demonstrated to enhance insulin sensitivity in cases of obesity by safeguarding β cells of the pancreatic gland from programmed cell death and facilitating the browning of peripheral white adipose tissue. Furthermore, the levels of IL-22 in serum have been interrelated with obesity; however, the findings from various studies have been incongruous.[52] As a result, research on the role of IL-22 has stimulated interest in endocrine metabolic diseases.[53] Significantly, the widespread persistent presence of elevated levels of circulating cytokines in PCOS has led to multiple studies suggesting that IL-22 can alleviate the illness by increasing systemic inflammation levels and decreasing insulin resistance.[54,55] Our research team has previously shown that IL-22 treatment in many PCOS mouse models alleviated insulin resistance and enhanced ovarian function.[52] Nonetheless, the direct influence of IL-22 on the ovary and its efficacy in treating non-metabolic PCOS patients remains ambiguous.

PCOS exhibits significant heterogeneity and includes multiple subgroups. Approximately 21-23% of women with PCOS predominantly exhibit ovarian dysfunction without associated metabolic problems, for whom current therapies are limited and non-specific. A prior study identified IL-22 as a component that facilitates the initiation of browning in white adipose tissue intensifies insulin resistance in polycystic ovary syndrome.[21] However, all PCOS models utilized in earlier research exhibited considerable metabolic disorders and do not indicate that IL-22 has the potential to improve the reproductive phenotype in non-metabolic PCOS patients.[56] This underscores the difficulties in creating non-metabolic PCOS models, complicating the development of a strategy that guarantees PCOS mice exhibit no metabolic symptoms. Certain research suggests the letrozole-induced polycystic ovary syndrome model may demonstrate a reproductive phenotype akin to PCOS without the presence of insulin resistance; however, the results seem ambiguous, as this model reveals contradictory metabolic traits.[57,58] Consequently, there is an immediate necessity for a dependable model of non-metabolic PCOS to examine the role of IL-22 on ovarian physiological function of ovarian activity within this framework.

An ROC curve analysis was conducted to assess the predicted capacity of PCOS. Adipisin was recognized as the most efficacious diagnostic parameter for PCOS (AUC=0.8518), demonstrating a sensitivity of 81% and a specificity of 97%. This was followed by lipocalin-2 (AUC=0. 8108), angiopoietin-2 (AUC=0. 7476), and IL-22 (AUC=0. 7476). Both lipocalin-2 and adipisin displayed superior diagnostic capacity for PCOS (AUC=0.8518 and AUC=0.8103, respectively), while angiopoietin-2 and IL-22 showed some diagnostic value for PCOS (AUC=0.7476 and AUC=0.7340, respectively). Notably, the combined diagnostic application of proinflammatory factors and adipokines enhanced the diagnostic accuracy for PCOS.

The Pearson correlation analysis indicated a substantial positive association among the studied parameters. Alterations in adipocyte activity influence the secretion of adipokines, proinflammatory substances originating from adipose tissue that elevate the risk of low-grade inflammation. The hormonal dysregulations linked to PCOS result in a persistent inflammatory response. The interaction between the ovarian microenvironment and visceral adipocytes may be influenced by circulating immune cells and inflammatory cytokines. The management of standard follicular maturation and ovulation is overseen by a precisely orchestrated inflammatory mechanism, which is perturbed in PCOS. Granulosa cells' production of inflammasome complexes is evidence of the inflammatory disruption linked to PCOS. As a result, it persists as an independent yet unverified hypothesis that immunological dysfunction directly leads to ovulatory failure.

Conclusions

This study discovered that patients with PCOS had elevated levels of all proinflammatory factors and adipokines compared to healthy women, indicating possible associations between these molecules and PCOS. Additionally, lipocalin-2, adipsin, angiopoietin-2, and IL-22 may serve as novel diagnostic biomarkers for PCOS. These results emphasize the possible contributions of proinflammatory elements and adipokines in PCOS and its associated metabolic alterations. However, additional investigation is required to thoroughly understand their mechanisms and implications in clinical practice.

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Online supplementary material:

Supplementary Table 1. Mean concentration of adipokines in serum samples from control and patient groups.

Supplementary Table 2. Serum proinflammatory factors concentration comparison between the patient and control groups.

Supplementary Figure 1. Receiver operating characteristic curve results of serum lipocalin-2.

Supplementary Figure 2. Receiver operating characteristic curve results of serum adipisin.

Supplementary Figure 3. Receiver operating characteristic curve results of serum angiopoietin-2.

Supplementary Figure 4. Receiver operating characteristic curve results of serum interleukin-22.

Supplementary Figure 5. A scatterplot shows the relationship between lipocalin-2 to adipsin in the polycystic ovarian syndrome group.

Supplementary Figure 6. A scatterplot shows the relationship between lipocalin-2 and interleukin-22 in the polycystic ovarian syndrome group.

Supplementary Figure 7. A scatterplot shows the relationship between lipocalin-2 and angiopoietin-2 in the polycystic ovarian syndrome group.

Supplementary Figure 8. A scatterplot shows the relationship between angiopoietin-2 and interleukin-22 in in the polycystic ovarian syndrome group.

Supplementary Figure 9. A scatterplot shows the relationship between angiopoietin-2 and adipsin in the polycystic ovarian syndrome group.