Journal of Medical and oral biosciences
ISSN (Online): 3007-9551
ISSN (Print): 3007-9543

JMOB
Open Access DOAJ

IRAQI Academic Scientific Journals

Type: Research article
Publish online: 30 / 05 / 2026

Comparative Analysis of Traditional and Emerging Salivary Biomarkers in Polycystic Ovary Syndrome Diagnosis

Lamyaa Kadhim Ouda[1]*

[1] Department of Pathological Analysis, College of Science, Thi-Qar University, Thi-Qar, Iraq
ORCID: https://orcid.org/0009-0005-1636-9472
Email address: lamyaa.kadhim@sci.utq.edu.iq

OPEN ACCESS

ARTICLE INFO

Received: 07 / 02 /2026
Revised: 27 / 02/ 2026
Accepted: 02 / 03 / 2026
Publish online: 30 / 05/ 2026
Plagiarism percentages at publication:13 %

* Corresponding Author: Lamyaa Kadhim Ouda
Email: lamyaa.kadhim@sci.utq.edu.iq

CITATION

Lamyaa Kadhim Ouda. (2026). Comparative Analysis of Traditional and Emerging Salivary Biomarkers in Polycystic Ovary Syndrome Diagnosis. JMOB. 3;(2): 01-14.
https://doi.org/10.58564/jmob.145

Abstract

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age globally, with a reported prevalence ranging from 10% to 20% depending on the diagnostic criteria used. This study designed to evaluate the differences in hormonal, metabolic, and inflammatory profiles, and to assess the diagnostic utility of non-invasive salivary cytokines (IL-6, TNF-α, IL-1β) in women with Polycystic Ovary Syndrome (PCOS). A case-control study was conducted on 100 women (50 PCOS and 50 controls). PCOS women were diagnosed based on the Rotterdam Criteria. Fasting blood and unstimulated saliva samples were collected. Biomarkers, including Total Testosterone, SHBG, FAI, HOMA-IR, and salivary IL-6, TNF-α, and IL-1β, were measured. Statistical analysis included Independent Samples t-test, Pearson's correlation, ROC curve analysis to determine diagnostic performance, and binary logistic regression to identify independent predictors. The PCOS group showed highly significant elevations ($P<0.001$) in all measured biomarkers compared to controls, with large effect sizes (Cohen's $d > 1.3$). Markedly, salivary IL-6, TNF-α, and IL-1β were substantially increased ($d > 2.4$). ROC analysis revealed exceptional diagnostic accuracy for the Free Androgen Index (FAI) (AUC=0.97) and the salivary cytokines, particularly salivary IL-1β (AUC=0.98) and salivary TNF-α (AUC=0.97). Binary logistic regression identified the FAI as the strongest independent predictor of PCOS (OR = 7.469, P=0.0121). In conclusion, the results of the current study showed that PCOS was strongly associated with a pronounced state of chronic low-grade inflammation, reliably reflected by salivary cytokines. The high diagnostic accuracy of salivary IL-1β and TNF-α suggests that non-invasive salivary analysis can serve as a highly accurate and patient-friendly tool for assessing the inflammatory component of PCOS, complementing the established role of the Free Androgen Index.

Keywords: PCOS, Salivary Cytokines, (FAI), Inflammation, Diagnostic Biomarkers.

Introduction

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age globally, with a reported prevalence ranging from 10% to 20% depending on the diagnostic criteria used (1). This heterogeneous condition

is characterized by a complex interplay of reproductive, metabolic, and psychological disturbances, leading to clinical manifestations such as oligo- or anovulation, hyperandrogenism (clinical or biochemical), and polycystic ovarian morphology (2). Beyond its reproductive consequences, PCOS is a significant public health concern due to its strong association with long-term metabolic comorbidities, including insulin resistance (IR), type 2 diabetes mellitus, dyslipidemia, and increased cardiovascular risk (3). The core pathophysiology of PCOS is driven primarily by two interconnected factors: hyperandrogenism and insulin resistance (4). Hyperandrogenism, often measured by the Free Androgen Index (FAI), is a hallmark of the syndrome, resulting from increased ovarian and adrenal androgen production. Insulin resistance, which is highly prevalent in both obese and lean women with PCOS, exacerbates hyperandrogenism by stimulating the production of androgens in the theca cells and simultaneously suppressing the hepatic synthesis of Sex Hormone-Binding Globulin (SHBG), thereby increasing the concentration of bioavailable androgens (5). The assessment of these hormonal and metabolic markers is fundamental to both the diagnosis and the management of the syndrome. Previous publication showed that the paradigm of PCOS has expanded to include chronic low-grade inflammation as a critical component of its etiology and pathogenesis (6). Numerous studies have demonstrated elevated levels of pro-inflammatory cytokines, such as Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-1 beta (IL-1β), in the serum of women with PCOS (7). This systemic inflammatory state is believed to contribute to insulin resistance, endothelial dysfunction, and the overall progression of metabolic complications (8).

Consequently, these inflammatory markers have emerged as potential targets for therapeutic intervention and as valuable biomarkers of disease severity (8). Despite the established diagnostic criteria, there remains a significant need for accurate, cost-effective, and non-invasive diagnostic tools (9). Traditional blood sampling for hormonal and metabolic profiles is invasive, stressful for patients, and subject to diurnal variations. Saliva, as a biological fluid, offers a promising alternative, as it contains a wide array of biomarkers, including hormones and cytokines that reflect their systemic concentrations (10, 11). The potential of salivary biomarkers, particularly salivary cytokines, to serve as reliable, non-invasive surrogates for systemic inflammation and hormonal status in PCOS has only recently begun to be explored (12).

Therefore, the primary objectives of the current study were to compare the levels of key hormonal, metabolic, and salivary inflammatory biomarkers (TNF-α, IL-6, IL-1β) between women with PCOS and healthy controls; to evaluate the diagnostic performance of these biomarkers, including the novel salivary cytokines, using Receiver Operating Characteristic (ROC) curve analysis; and (3) to identify the independent predictors of PCOS using binary logistic regression analysis. By rigorously assessing the diagnostic utility of these non-invasive salivary markers, this study aims to contribute to the development of more practical and patient-friendly diagnostic strategies for PCOS.

Materials and Methods

Ethical approval

This study adhered to the ethical principles of the Declaration of Helsinki and received approval from the relevant ethics committee (Ref. No. 5-61, dated 18/09/2024).

Study Design and Participants

This was a case-control study involving 100 female participants of reproductive age (20–35 years), divided equally into two groups: the Polycystic Ovary Syndrome (PCOS) group (n=50) and the healthy control group (n=50). Participants were recruited from the outpatient gynecology clinics of the Bint Al-Huda Maternity and Children Teaching Hospital and Al-Hussein Teaching Hospital in Nasiriyah, Thi-Qar, Iraq, between September 2024 and September 2025.

Inclusion Criteria: Diagnosis was established according to the Rotterdam Consensus Criteria (2003), requiring the presence of at least two of the following three features(13):

1. Oligo- or anovulation (menstrual cycles > 35 days or < 8 cycles per year).
2. Clinical hyperandrogenism (e.g., hirsutism, assessed by the modified Ferriman-Gallwey (mFG) score > 8) and/or biochemical hyperandrogenism (elevated Free Androgen Index).
3. Polycystic ovarian morphology (PCOM) on ultrasound (≥ 20 follicles measuring 2–9 mm in diameter and/or an ovarian volume > 10 mL in at least one ovary).

Exclusion Criteria: Participants were excluded if they had any other endocrine disorders (e.g., congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, thyroid dysfunction), were pregnant, had taken hormonal medications (including oral contraceptives) or insulin-sensitizing agents (e.g., metformin) within the preceding three months, or had any acute inflammatory conditions.

Sample Collection and Processing

Blood Sample Collection: Blood samples were collected from all participants after a 10–12 hours overnight fast, typically between 8:00 AM and 10:00 AM to minimize diurnal variation. Serum was separated by centrifugation at 3000 rpm for 15 minutes, aliquoted, and stored at –80°C until biochemical analysis.

Saliva Sample Collection: Unstimulated whole saliva samples were collected using the passive drool method, following standardized protocols(14). Participants were instructed to refrain from eating, drinking (except water), smoking, and oral hygiene procedures for at least 60 minutes prior to collection. Saliva was collected into sterile polypropylene tubes for 5 minutes. Samples were immediately centrifuged at 10,000 rpm for 10 minutes at 4°C to remove cellular debris, aliquoted, and stored at –80°C until cytokine analysis.

Sample Collection and Processing

Blood Sample Collection: Blood samples were collected from all participants after a 10–12 hour overnight fast, typically between 8:00 AM and 10:00 AM to minimize diurnal variation. Serum was separated by centrifugation at 3000 rpm for 15 minutes, aliquoted, and stored at –80°C until biochemical analysis. Saliva Sample Collection: Unstimulated whole saliva samples were collected using the passive drool method, following standardized protocols(14). Participants were instructed to refrain from eating, drinking (except water), smoking, and oral hygiene procedures for at least 60 minutes prior to collection. Saliva was collected into sterile polypropylene tubes for 5 minutes. Samples were immediately centrifuged at 10,000 rpm for 10 minutes at 4°C to remove cellular debris, aliquoted, and stored at –80°C until cytokine analysis.

Biochemical and Hormonal Assays

All biochemical and hormonal parameters were measured using commercially available, validated assay kits.

1. Fasting glucose was determined using the standard hexokinase method with a reagent kit from Roche Diagnostics (Cat. No. 05168791190; Mannheim, Germany) on a cobas series analyzer.
2. Fasting insulin and Sex Hormone-Binding Globulin (SHBG) were quantified using a validated electrochemiluminescence immunoassay (ECLIA) on a cobas e series analyzer (Roche Diagnostics, Mannheim, Germany).Specifically, the Elecsys Insulin assay (Cat. No. 12017547122) and the Elecsys SHBG assay (Cat. No. 03052001190) were utilized.
3. Total Testosterone was measured using a high-sensitivity competitive binding ELISA kit (DRG International, Inc., Cat. No. EIA-1559; Springfield, NJ, USA). The Free Androgen Index (FAI) was calculated using the standard formula: FAI = [Total Testosterone (nmol/L) / SHBG (nmol/L)] × 100(15).
4. Insulin resistance was assessed using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index, calculated as: HOMA-IR = [Fasting Glucose (mmol/L) × Fasting Insulin (μU/mL)] / 22.5(16).
5. The salivary concentrations of the pro-inflammatory cytokines Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), and Interleukin-1 beta (IL-1β) were determined using highly sensitive, commercially available sandwich ELISA kits specifically validated for use with human saliva. The measurements were performed using the following kits: Salivary IL-6 was quantified using the Salimetrics Salivary IL-6 ELISA Kit (Cat. No. 1-3602; Salimetrics, LLC, Carlsbad, CA, USA). For salivary TNF-α, the Human TNF-alpha ELISA Kit from MP Biomedicals (Cat. No. 08L100043; Solon, OH, USA), which is validated for saliva samples, was used. Salivary IL-1β was measured using the Human IL-1 beta/IL-1F2 Quantikine HS ELISA Kit (Cat. No. HSLB00D; Bio-Techne/R&D Systems, Inc., Minneapolis, MN, USA), which is validated for saliva samples. All assays were performed according to the respective manufacturer's instructions. All samples were run in duplicate, and the intra- and inter-assay coefficients of variation were maintained below 10% to ensure high data quality and reproducibility.

Statistical Analysis

Statistical analysis was conducted using [SPSS version 26.0]. Descriptive statistics were presented as Mean ± Standard Deviation (Mean ± SD). Group comparisons were performed using the Independent Samples t-test, and the magnitude of the difference was quantified using Cohen's d effect size. The relationships between biomarkers were explored using Pearson's correlation coefficient (r). The diagnostic utility of the biomarkers was assessed via Receiver Operating Characteristic (ROC) curve analysis, reporting the Area Under the Curve (AUC) and its 95% Confidence Interval (CI). Finally, Binary Logistic Regression was employed to identify independent predictors of PCOS, with model fit assessed by the Nagelkerke R^2. A two-sided P-value of < 0.05 was considered statistically significant for all analyses.

Results

Table 1, present statistical comparison between the Polycystic Ovary Syndrome (PCOS) group and the Control group demonstrated excellent matching in Age (P=0.506), confirming the demographic homogeneity of the groups. However, the results revealed highly significant statistical differences (P<0.001) across all other evaluated biomarkers, accompanied by very large effect sizes (Cohen's d up to 3.113). The PCOS group exhibited significantly elevated indicators of obesity (BMI), insulin resistance (HOMA-IR and Fasting Insulin), and hyperandrogenism (Total Testosterone and Free Androgen Index), alongside a marked reduction in SHBG. Crucially, all measured salivary inflammatory markers (TNF-α, IL-6, IL-1β) were substantially increased in the PCOS group, with effect sizes exceeding 2.4, strongly confirming a systemic inflammatory state associated with the syndrome.

Table. 1: Baseline Characteristics and Comparative Analysis of Key Biomarkers between Polycystic Ovary Syndrome Patients and Control Group

Biomarker	PCOS (50) Mean ± SD	Control (50) Mean ± SD	P-Value	Cohen's d
Age	25.50 ± 3.75	25.87 ± 3.50	0.506	-0.103
BMI	27.80 ± 5.08	22.25 ± 2.68	<0.001	1.368
mFG Score	12.52 ± 3.30	4.08 ± 1.96	<0.001	3.113
Fasting Glucose	90.26 ± 6.19	85.91 ± 4.92	<0.001	0.778
Fasting Insulin	14.76 ± 5.35	7.81 ± 3.20	<0.001	1.575
Total Testosterone	61.12 ± 13.55	35.28 ± 9.30	<0.001	2.223
SHBG	35.32 ± 11.19	60.24 ± 15.36	<0.001	-1.855
AMH	7.54 ± 2.88	3.23 ± 1.44	<0.001	1.894
Alpha-1 Antitrypsin	2.25 ± 0.37	1.60 ± 0.28	<0.001	1.966
CRP/Albumin Ratio	2.88 ± 1.12	1.50 ± 0.44	<0.001	1.620
Salivary TNF-α	17.52 ± 2.38	11.99 ± 1.39	<0.001	2.835
Salivary IL-6	10.00 ± 2.07	5.86 ± 1.17	<0.001	2.462
Salivary IL-1β	171.23 ± 20.22	127.55 ± 15.01	<0.001	2.454
HOMA-IR	3.29 ± 1.25	1.67 ± 0.74	<0.001	1.588
Free Androgen Index	7.07 ± 4.65	2.18 ± 0.91	<0.001	1.461

 # Data are presented as Mean ± Standard Deviation (Mean ± SD). Group comparisons were performed using the Independent Samples t-test. A two-sided P-value < 0.05 was considered statistically significant. Cohen's d was calculated to determine the effect size of the differences between the groups. Abbreviations: PCOS, Polycystic Ovary Syndrome; BMI, Body Mass Index; mFG, modified Ferriman-Gallwey; SHBG, Sex Hormone-Binding Globulin; AMH, Anti-Müllerian Hormone; CRP, C-Reactive Protein; TNF-α, Tumor Necrosis Factor-alpha; IL-6, Interleukin-6; IL-1β, Interleukin-1 beta; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

Table. 2 presents the ROC curve analysis results, evaluating the diagnostic performance of selected biomarkers in discriminating between PCOS cases and controls. Overall, all biomarkers exhibited excellent diagnostic utility, with Area Under the Curve (AUC)

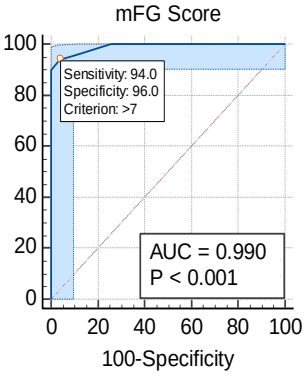
values exceeding 0.90. The mFG Score emerged as the top-performing indicator with the highest AUC of 0.99 (95% CI: 0.97, 1.00) and the highest Accuracy (0.90), suggesting near-perfect discriminatory power. Notably, the salivary inflammatory markers, specifically Salivary IL-1β (AUC=0.98) and Salivary TNF-α (AUC=0.97), demonstrated exceptional diagnostic performance comparable to the Free Androgen Index (AUC=0.97). These markers consistently showed high Sensitivity and Specificity, mostly exceeding 0.90, underscoring their potential as robust and reliable diagnostic tools for PCOS when utilizing the determined optimal thresholds (Figure.1).

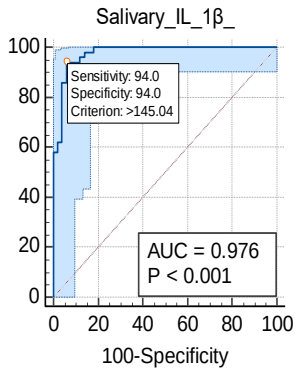
Table. 2: Diagnostic Performance of Selected Biomarkers for Identifying Polycystic Ovary Syndrome Based on Receiver Operating Characteristic (ROC) Curve Analysis

.

Biomarker	AUC95% CI	Optimal Threshold	Sens*	Speci*	PPV	NPV	Accuracy
mFG Score	0.99 [0.97, 1.00]	>7	0.94	0.96	0.96	0.96	0.90
Salivary IL-1β	0.98 [0.95, 1.00]	>145.52	0.94	0.94	0.92	0.96	0.88
Salivary TNF-α	0.97[0.95, 1.00]	>13.76	0.96	0.96	0.94	0.94	0.88
Salivary IL-6	0.95[0.89, 0.98]	>7.29	0.90	0.97	0.94	0.94	0.84
Free Androgen Index	0.97[0.95, 0.99]	>3.15	0.96	0.92	0.92	0.92	0.88
SHBG	0.91[0.74, 0.96]	≤50.93	0.92	0.88	0.88	0.92	0.70
Alpha-1 Antitrypsin	0.92[0.85, 0.97]	>1.93	0.84	0.88	0.84	0.89	0.72

 # Diagnostic performance was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The optimal threshold for each biomarker was determined as the value that maximizes the Youden's index (Sensitivity + Specificity - 1). Abbreviations: AUC, Area Under the Curve; CI, Confidence Interval; Sens, Sensitivity; Speci, Specificity; PPV, Positive Predictive Value; NPV, Negative Predictive Value; mFG, modified Ferriman-Gallwey; IL-1β, Interleukin-1 beta; TNF-α, Tumor Necrosis Factor-alpha; IL-6, Interleukin-6; SHBG, Sex Hormone-Binding Globulin.





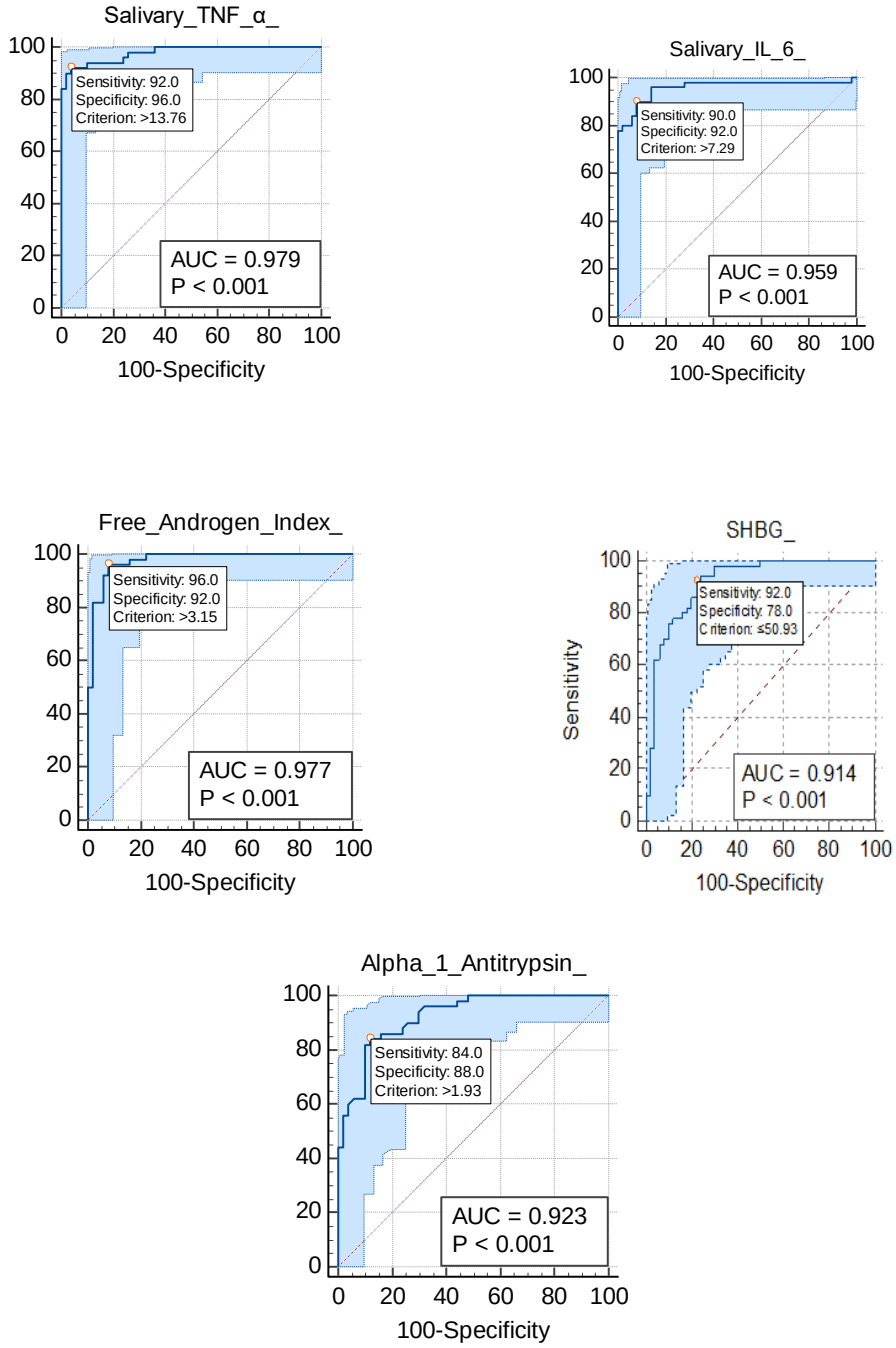


Figure. 1: Shows the diagnostic Accuracy of Key Biomarkers for Polycystic Ovary Syndrome Receiver Operating Characteristic (ROC) curves. It illustrating the diagnostic performance of: (A) modified Ferriman-Gallwey (mFG) Score, (B) Salivary Interleukin-1 beta (IL-1β), (C) Salivary Tumor Necrosis Factor-alpha (TNF-α), (D) Salivary Interleukin-6 (IL-6), (E) Free Androgen Index (FAI), and (F) Alpha-1 Antitrypsin in discriminating between women with Polycystic Ovary Syndrome (PCOS) and healthy controls. For each biomarker, the Area Under the Curve (AUC), the optimal diagnostic threshold (Criterion), and the corresponding Sensitivity and Specificity are provided. All AUC values were statistically significant (P < 0.001).

Table. 3 illustrates the correlation matrix, highlighting the interrelationships within the pathogenic pathways of PCOS. The results confirmed a strong and statistically significant negative correlation between the Free Androgen Index (FAI) and Sex Hormone-Binding Globulin (SHBG) ($r = -0.740$, $P < 0.001$), validating the known physiological relationship. A significant positive correlation was observed between Salivary IL-1β and Total Testosterone ($r = 0.294$, $P = 0.038$), suggesting that elevated androgen levels may be linked to increased inflammatory activity. Furthermore, a significant positive correlation between the mFG Score and the CRP/Albumin Ratio ($r = 0.334$, $P = 0.017$) was noted, linking the severity of clinical symptoms to the chronic inflammatory state. Collectively, the correlation matrix indicates that salivary inflammatory markers exhibit moderate to strong associations with key hormonal and metabolic indicators, supporting the role of inflammation as a co-factor in PCOS pathogenesis.

Table. 3: Correlation Matrix Illustrating the Interrelationships among Hormonal, Metabolic, and Inflammatory Biomarkers.

	Total_Testosterone_	Salivary_IL_1β_	mFG Score	Salivary_IL_6_	HOMA_IR	Anti_Müllerian_Hormone_	Salivary_TNF_α_	CRP_Albumin_Ratio_	Alpha_1_Antitrypsin_	Free_Androgen_Index_
Total Testosterone	1									
Salivary IL1β	0.010 P=0.9435	1								
mFG Score	-0.096 P=0.5075	-0.030 P=0.8336	1							
Salivary IL6	-0.181 P=0.2092	0.294 P=0.0382	0.141 P=0.3279	1						
HOMA_IR	-0.059 P=0.6828	0.089 P=0.5376	-0.102 P=0.4802	0.125 P=0.3880	1					
Anti Müllerian Hormone	0.065 P=0.6561	0.136 P=0.3478	0.209 P=0.1446	-0.126 P=0.3820	0.059 P=0.6830	1				
Salivary TNF α	0.252 P=0.0770	-0.086 P=0.5546	0.032 P=0.8246	-0.049 P=0.7370	0.021 P=0.8873	-0.062 P=0.6685	1			
CRP Albumin Ratio_	-0.207 P=0.1490	-0.085 P=0.5573	0.334 P=0.0178	0.163 P=0.2580	-0.091 P=0.5318	0.190 P=0.1863	-0.244 P=0.0882	1		
Alpha_1 Antitrypsin	-0.110 P=0.4453	0.001 P=0.9936	-0.217 P=0.1303	0.019 P=0.8964	0.097 P=0.5013	-0.256 P=0.0724	-0.079 P=0.5833	-0.249 P=0.0808	1	
Free Androgen Index	0.519 P=0.0001	-0.055 P=0.7044	-0.005 P=0.9741	-0.270 P=0.0584	-0.076 P=0.5998	0.151 P=0.2957	0.150 P=0.2998	-0.307 P=0.0303	-0.031 P=0.8323	1
SHBG	-0.217 P=0.1297	0.103 P=0.4756	-0.103 P=0.4775	0.231 P=0.1071	0.052 P=0.7180	-0.200 P=0.1636	-0.093 P=0.5196	0.282 P=0.0474	0.047 P=0.7473	-0.740 P<0.0001

 # Correlations among biomarkers were assessed using Pearson's correlation coefficient (r). The upper value in each cell represents the correlation coefficient (r), and the lower value represents the corresponding P-value. A two-sided P-value < 0.05 was considered statistically significant. Significant correlations are highlighted for clarity. Abbreviations: mFG, modified Ferriman-Gallwey; SHBG, Sex

Hormone-Binding Globulin; CRP, C - reactive protein; TNF-α, Tumor Necrosis Factor-alpha; IL-6, Interleukin-6; IL-1β, Interleukin-1 beta; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

Table. 4 presents the results of the binary logistic regression model, which aimed to identify independent biomarkers predicting the presence of PCOS. The model demonstrated high explanatory power, evidenced by the Cox & Snell R^2 (0.7209) and Nagelkerke R^2 (0.9612) values, and achieved overall statistical significance ($P < 0.0001$). The analysis revealed that the Free Androgen Index (FAI) was the most significant independent predictor, with a one-unit increase associated with a 7.469-fold increase in the odds of having PCOS (95% CI: 1.552 - 35.946, P=0.0121). Salivary IL-6 also showed a marginal association (P=0.0506), with a 17.727-fold increase in odds for PCOS per unit increase, although its 95% confidence interval nearly touches unity (0.991 - 316.809). These findings strongly suggest that hyperandrogenism, as measured by FAI, is the primary independent determinant of PCOS in this model, with a potential, albeit less clear, role for salivary inflammatory markers.

Table. 4: Independent Predictors of Polycystic Ovary Syndrome (PCOS) Identified by Binary Logistic Regression Analysis.

(Variable)	(Coefficient)	(Std. Error)	Wald	P	(Odds Ratio)	95% (95% CI)
Salivary_IL_6_	2.87507	1.47103	3.8199	0.0506	17.7267	0.991 to 316.809
Free_Androgen_Index_	2.01078	0.80165	6.2916	0.0121	7.4692	1.552 to 35.946
Constant	-29.61022	13.58282	4.7523	0.0293	-	-
mFG_Score	non	non	non	non	non	non
Total_Testosterone_	non	non	non	non	non	non
SHBG_	non	non	non	non	non	non
Alpha_1_Antitrypsin_	non	non	non	non	non	non
Salivary_IL_1β_	non	non	non	non	non	non
Salivary_TNF_α_	non	non	non	non	non	non
HOMA_IR	non	non	non	non	non	non
CRP_Albumin_Ratio_	non	non	non	non	non	non
Anti_Müllerian_Hormone_	non	non	non	non	non	non

Significance level	P < 0.0001
Cox & Snell R^2	0.7209
Nagelkerke R^2	0.9612

 # Binary logistic regression analysis (enter method) was performed to identify independent predictors of PCOS. The model's goodness-of-fit was assessed using the Cox & Snell R^2 and Nagelkerke R^2 values. The overall model was statistically significant ($P < 0.0001$). Variables with "non" were not entered into the final step of the model due to lack of significance in univariate analysis or multicollinearity.
Abbreviations: Std. Error, Standard Error; CI, Confidence Interval.

Discussion

The present study aimed to investigate the differences in hormonal, metabolic, and inflammatory biomarkers between women with Polycystic Ovary Syndrome (PCOS) and

healthy controls, to assess the diagnostic utility of these markers, and to identify the independent predictors of the syndrome. The findings of the current study, summarized in Table 1, confirmed that the established clinical and biochemical profile of PCOS, showed significant elevations in markers of hyperandrogenism, insulin resistance, and chronic low-grade inflammation. It is worth to mention, that this study provided novel evidence regarding the significant elevation and high diagnostic utility of salivary inflammatory cytokines in PCOS, highlighting their potential as non-invasive biomarkers. The main finding of this research is the highly significant increase in all measured salivary inflammatory markers—Salivary TNF-α, IL-6, and IL-1β—in the PCOS group compared to controls (Table 1). The effect sizes (Cohen's d > 2.4) were exceptionally large, underscoring the profound inflammatory state associated with the syndrome. This observation compatible with previous publication that recognizes PCOS not merely as an endocrine disorder but as a condition rooted in chronic, low-grade inflammation(17). The elevated levels of these pro-inflammatory cytokines, particularly IL-6, are known to be implicated in insulin resistance and hyperandrogenism, creating a vicious cycle that drives the pathogenesis of PCOS (7).

The current results extend previous findings by demonstrating that this systemic inflammation is reliably reflected in saliva. Studies have shown that salivary cytokine levels, including IL-6 and TNF-α, correlate significantly with their serum counterparts, validating saliva as a viable, non-invasive medium for monitoring systemic inflammatory status(18, 19). The strong positive correlation observed between Salivary IL-1β and Total Testosterone (Table 3) further supports the hypothesis that the inflammatory burden is directly linked to the degree of hyperandrogenism, suggesting a potential mechanism where inflammatory signals may modulate ovarian steroidogenesis or alter androgen metabolism(20).

Consistent with the Rotterdam criteria and established pathophysiology, these analysis confirmed the central role of hyperandrogenism. The Free Androgen Index (FAI) was significantly elevated in the PCOS group (Table 1) and emerged as the most robust independent predictor of PCOS in the binary logistic regression model (Table 4). A one-unit increase in FAI was associated with a 7.469-fold increase in the odds of having PCOS (P=0.0121). This finding reinforces the concept that the bioavailable androgen fraction is the most critical driver of the clinical phenotype. Furthermore, the strong negative correlation between FAI and SHBG ($r = -0.740$, $P < 0.001$) shown in Table 3 is a fundamental physiological observation, confirming that reduced SHBG levels contribute significantly to the increased free androgen pool and, consequently, the clinical manifestation of hyperandrogenism(21).

While Salivary IL-6 showed a marginal association in the regression model (P=0.0506), the overwhelming predictive power of FAI suggests that, in this group, hyperandrogenism remains the primary diagnostic and predictive factor, with inflammation potentially acting as a strong co-factor or consequence rather than the sole independent cause.

The ROC curve analysis (Table 2) provides compelling evidence for the diagnostic potential of several biomarkers. The Modified Ferriman-Gallwey (mFG) (AUC=0.99) and the Free Androgen Index (AUC=0.97) demonstrated near-perfect discriminatory power, which is expected given their role in the diagnostic criteria. More importantly, the exceptional performance of the salivary markers, particularly Salivary IL-1β (AUC=0.98) and Salivary TNF-α (AUC=0.97), suggests that these non-invasive measurements can serve as highly accurate screening or monitoring tools. The high sensitivity and specificity values (all >0.90 for these key markers) indicate that salivary testing could offer a practical, stress-free alternative to traditional blood sampling for assessing both the

inflammatory status and the presence of PCOS . This is particularly relevant for longitudinal studies or in populations where blood sampling is challenging.

Clinical implications

The findings of this study carry some important clinical implications. First, the high diagnostic accuracy of salivary IL-1β and TNF-α suggests that a simple, non-invasive saliva test could potentially be developed as a first-line screening tool for PCOS, especially in primary care settings. This approach may also be useful for monitoring treatment efficacy over time, reducing the need for venipuncture and improving patient comfort. In addition, the strong evidence of systemic inflammation reflected by these salivary cytokines reinforces the importance of incorporating anti-inflammatory therapeutic strategies into PCOS management, alongside the traditional hormonal and metabolic interventions. Furthermore, because inflammatory markers show a strong correlation with metabolic dysfunction, these salivary biomarkers may have value in risk stratification by identifying PCOS patients who are at higher risk for long-term complications such as type 2 diabetes and cardiovascular disease.

Limitations

The limitation of the current study was not establish causality between elevated biomarkers and PCOS; therefore, longitudinal studies are needed to determine whether increased salivary cytokines appear before or after the development of the syndrome. Although the findings were statistically significant, the relatively limited sample size suggests that larger, multi-center studies are necessary to validate the diagnostic thresholds identified through ROC analysis. Moreover, absence of paired serum cytokine measurements limits the ability to directly correlate salivary inflammatory marker levels with systemic circulation, even though previous literature suggests such correlations exist.

Conclusion

The results of the following study revealed a relationship between the levels of salivary biomarkers and the hormonal, metabolic, and inflammatory profiles of women with Polycystic Ovary Syndrome (PCOS). Moreover, the findings confirmed that the PCOS group exhibits significant elevations in markers of hyperandrogenism and insulin resistance, alongside a pronounced state of chronic low-grade inflammation, as evidenced by the substantial increase in salivary TNF-α, IL-6, and IL-1β. These findings confirm that the PCOS group exhibits significant elevations in markers of hyperandrogenism and insulin resistance, alongside a pronounced state of chronic low-grade inflammation, as evidenced by the substantial increase in salivary TNF-α, IL-6, and IL-1β. Prominently, the Free Androgen Index (FAI) was identified as the strongest independent predictor of PCOS. However, the salivary inflammatory cytokines (IL-1β and TNF-α) demonstrated an exceptional diagnostic performance (AUC > 0.97), comparable to the FAI. This strongly suggests that salivary analysis offers a highly accurate, non-invasive, and patient-friendly method for assessing the inflammatory component of PCOS. Future research should focus on large-scale prospective studies to validate optimal cut-off values for salivary IL-1β and TNF-α as diagnostic markers for PCOS, along with intervention

trials assessing whether anti-inflammatory strategies improve cytokine levels and clinical outcomes. Additionally, mechanistic studies are needed to clarify the molecular links between salivary cytokines, ovarian dysfunction, and insulin signaling to support the development of targeted therapies.

Declarations

Acknowledgment

The author thanks the participants for their cooperation.

Ethics statement

Ethics statement: The authors declare that this study was conducted in accordance with the ethical standards and guidelines outlined in the journal's "Ethics Approval" section of the author guidelines. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Availability of Data and Materials

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

Competing Interests

The authors declare that they have no competing interests (financial or non-financial) that could have influenced the work reported in this paper.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Authors' Contributions

LKO: designed the study, performed the laboratory assays & the statistical analysis, and wrote the manuscript and was responsible for patient recruitment and sample collection,

References

1. Farhana AA, Shi J. A Narrative Review on Global Epidemiology of PCOS and its Hormonal Management. SSR Inst Int J Life Sci. 2024;10(3):5516-5521. doi: 10.21276/SSR-IIJLS.2024.10.3.17.

2. Tammo O. Understanding Polycystic Ovary Syndrome: A Comprehensive Guide [Internet]. Obstetrics and Gynecology. IntechOpen; 2025. https://doi.org/10.5772/intechopen.1010597

3. Kakoly NS, Moran LJ, Teede HJ, Joham AE. Cardiometabolic risks in PCOS: a review of the current state of knowledge. Expert Rev Endocrinol Metab. 2019 Jan;14(1):23-33. doi: 10.1080/17446651.2019.1556094. PMID: 30556433.

4. Herman R, Sikonja J, Jensterle M, Janez A, Dolzan V. Insulin Metabolism in Polycystic Ovary Syndrome: Secretion, Signaling, and Clearance. Int J Mol Sci. 2023 Feb 5;24(4):3140. doi: 10.3390/ijms24043140. PMID: 36834549; PMCID: PMC9962893.

5. Zeng X, Xie YJ, Liu YT, Long SL, Mo ZC. Polycystic ovarian syndrome: correlation between hyperandrogenism, insulin resistance and obesity. Clin Chim Acta. 2020 Mar;502:214-221. doi: 10.1016/j.cca.2019.11.003. PMID: 31733195.

6. Rudnicka E, Suchta K, Grymowicz M, Calik-Ksepka A, Smolarczyk K, Duszewska AM, Smolarczyk R, Meczekalski B. Chronic low grade inflammation in pathogenesis of PCOS. Int J Mol Sci. 2021 Apr 6;22(7):3789. doi: 10.3390/ijms22073789. PMID: 33917519; PMCID: PMC8038770.

7. Azizova ZS, Musakhodzhayeva DA, Ruzimurodov NF, Olimova NI. Systemic relationships of inflammatory, hormonal, and metabolic markers in women with polycystic ovary syndrome. Russian Journal of Immunology. 2025;28(4):1055-1060. doi: 10.46235/1028-7221-17224-SRO.

8. Faloia E, Michetti G, De Robertis M, Luconi MP, Furlani G, Boscaro M. Inflammation as a link between obesity and metabolic syndrome. J Nutr Metab. 2012;2012:476380. doi: 10.1155/2012/476380. PMID: 22523672; PMCID: PMC3317136.

9. Viardot-Foucault V. Enhancing the accuracy of polycystic ovary syndrome diagnosis. Ann Acad Med Singap. 2023 Dec 28;52(12):647-648. doi: 10.47102/annals-acadmedsg.2023397.

10. Ryznar R, Andrews N. Salivary Neuropeptides and Cytokines: Indicators of Stress and Resilience. In: Aitken-Saavedra J, Rojas-Alcayaga G, Braz-Silva PH, editors. Salivary Analysis - Biomarkers, Clinical Applications, and Emerging Technologies. London: IntechOpen; 2025. doi: 10.5772/intechopen.1010391.

11. Dagli N, Haque M, Kumar S. A Bibliometric Analysis of Clinical Trials on Salivary Biomarkers for Mental Health (2003-2024). Cureus. 2024 Jul 16;16(7):e64635. doi: 10.7759/cureus.64635. PMID: 39021745; PMCID: PMC11253590.

12. Yoshizawa JM, Schafer CA, Schafer JJ, Farrell JJ, Paster BJ, Wong DT. Salivary biomarkers: toward future clinical and diagnostic utilities. Clin Microbiol Rev. 2013 Oct;26(4):781-791. doi: 10.1128/CMR.00021-13. PMID: 24092855; PMCID: PMC3811231.

13. Panda S, Jyothi K, Konar L, Hussain Z. Unmasking the Overlooked Symptoms of PCOS: Navigating Ovulatory Dysfunction, Menstrual Disorders, and Acne. In: Wang Z, editor. Understanding Polycystic Ovary Syndrome - Symptoms, Diagnosis, and Treatment Options. IntechOpen; 2025. doi: 10.5772/intechopen.1011570.

14. Mortazavi H, Yousefi-Koma AA, Yousefi-Koma H. Extensive comparison of salivary collection, transportation, preparation, and storage methods: a systematic review. BMC Oral Health. 2024 Feb 2;24(1):168. doi: 10.1186/s12903-024-03902-w. PMID: 38308289; PMCID: PMC10837873.

15. Sanke S, Chander R, Garg T, Jain A. Free Androgen Index (FAI): Marker of Premature Androgenetic Alopecia in Men. J Microb Biochem Technol. 2016 Feb 24;8(2):97-99. doi: 10.4172/1948-5948.1000269.

16. Orbay E, Demiral BH, Tüzün S, Öner C. The association of insulin resistance with body mass index and body fat percentages in non-diabetic obese women. Anatol J Fam Med. 2018;1(1):13-16. doi: 10.5505/anatoljfm.2018.88598.

17. Deng H, Chen Y, Xing J, Zhang N, Xu L. Systematic low-grade chronic inflammation and intrinsic mechanisms in polycystic ovary syndrome. Front Immunol. 2024;15:1470283. doi: 10.3389/fimmu.2024.1470283. PMID: 39749338; PMCID: PMC11693511.

18. Çelik E, Kara SS, Çevik Ö. The potential use of saliva as a biofluid for systemic inflammatory response monitoring in children with pneumonia. Indian J Pediatr. 2022 May;89(5):477-483. doi: 10.1007/s12098-021-03973-5. PMID: 34595601.

19. Shakeeb N, Varkey P, Hynse A, Ajit A. Saliva as a Potential Specimen to Monitor IL-6, TNF-α and IL-10 in COVID-19 Patients. Inflammation. 2022 Dec;45(6):2368-2374. doi: 10.1007/s10753-022-01698-x. PMID: 35676607; PMCID: PMC9177134.

20. Velez LM, Seldin M, Motta AB. Inflammation and reproductive function in women with polycystic ovary syndrome. Biol Reprod. 2021 Jun 4;104(6):1205-17. doi: 10.1093/biolre/ioab050. PMID: 33739372; PMCID: PMC8785941.

21. Zapała B, Marszalec P, Piwowar M, Chmura O, Milewicz T. Reduction in the free androgen index in overweight women after sixty days of a low glycemic diet. Exp Clin Endocrinol Diabetes. 2024 Jan 18;132(1):6-14. doi: 10.1055/a-2201-8618. PMID: 38237611; PMCID: PMC10796197.