

Article

Assessment of Sirtuin-3 and Omentin-1 as Potential Biomarkers in Women with Polycystic Ovary Syndrome

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Abstract: Polycystic ovary syndrome (PCOS) has metabolic and oxidative characteristics, which has led to the exploration of circulating levels of SIRT3 and omentin-1 as candidate biochemical markers. Objective to assess erythrocyte glutathione peroxidase (GPx) activity and biochemical correlation in the serum SIRT3 and omentin-1 levels between women with and without PCOS. A hospital-based observational case-control study at Al-Shirqat General Hospital, Iraq, was conducted between 1–31 March 2026, involving 40 women with PCOS and 40 controls who were assessed by a specialist gynecologist. Group contrasts, adjusted regression analysis and intra-group Spearman correlations were used to assess immunoreactive proteins in serum as well as GPx activity in erythrocytes. Mean (SD) SIRT3 was 11.17 (2.28) versus 14.97 (3.15) ng/mL, omentin-1 was 31.41 (6.37) versus 46.76 (6.40) ng/mL, and erythrocyte GPx activity was 28.27 (5.50) versus 35.41 (6.07) U/g Hb for PCOS versus controls (all Welch $P < 0.001$). All pairwise within PCOS Spearman associations among the three indicators were not statistically significant. For IL-1 β , 78 of the 80 values recorded were below the lower quantitative range (3.9 pg/mL) specified for the assay identified; these values were not included in quantitative inferences. The three biochemical indices were different between groups; but the relationships within groups were not supported by these observations. Findings are descriptive, not causative or diagnostic.

Keywords: Biochemistry, Glutathione Peroxidase, Interleukin-1 Beta, Omentin-1, Polycystic Ovary Syndrome, SIRT3

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1. Introduction

Polycystic ovary syndrome (PCOS) is a complex syndrome associated with a cluster of reproductive abnormalities that occurs together, to varying extents, with insulin abnormalities, adipose tissue abnormalities, disturbances of oxidative processes and inflammation. While ovarian morphology plays a role in the diagnosis, diagnosis for PCOS as a purely ovarian problem misses the metabolic variety seen in those with the condition. The 2023 global evidence-based guideline concluded that there should be standardized diagnostic assessment, proper exclusion of alternative diagnosis and recognition of the influence of phenotype, age, and metabolic features on clinical presentation [1]. The most difficult part of biochemical investigation is determining what measurements to take that would reflect useful molecular processes instead of only statistically distinct laboratory values. A biomarker must have analytic validity, a biologically meaningful interpretation, and an easily repeatable relationship between the marker and the condition. A clinical utility claim is a much harder one to make than an observed difference between selected groups [2].

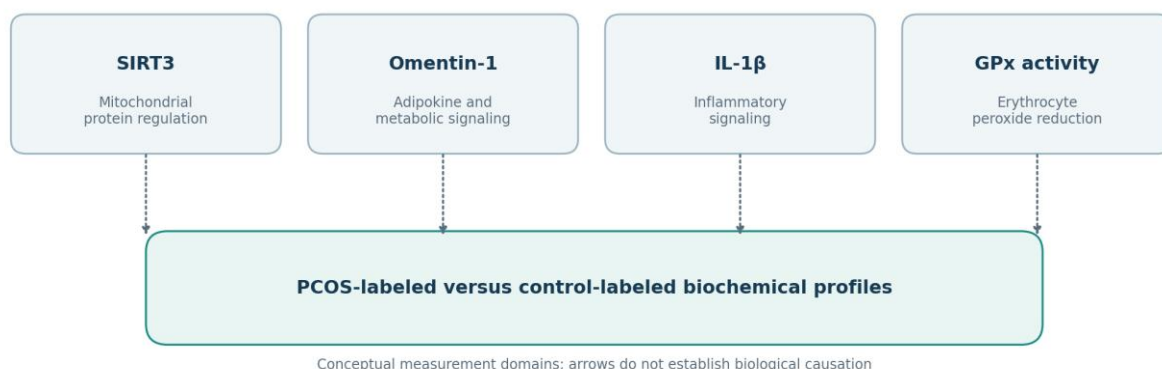


Figure 1. Biochemical domains motivating the study: mitochondrial regulation, adipokine signaling, inflammatory cytokines and antioxidant activity. Arrows denote conceptual hypotheses rather than established causal connections.

Subtle abnormalities in energy generation, substrate oxidation, antioxidant mechanisms, inflammatory pathways and communication between the adipose and other endocrine organs are all considered to be part of the biochemical environment of PCOS. These processes are overlapping and not mutually exclusive. One way to assess circulating inflammation is to measure it in the plasma cytokines, while another is to measure the activity of the enzymes within the erythrocytes. Similarly, the soluble concentration of an adipokine does not directly indicate insulin resistance, and the circulating concentration of a mitochondrial enzyme mainly present in the intracellular compartment does not directly reflect the amount of deacetylase activity in mitochondria. Compartmental, functional and measurement differences are particularly relevant when interpreting the results of multiple assays. Previous meta-analytical studies reported that markers of oxidative stress were often altered among women with PCOS, even after accounting for adiposity, but also revealed significant intermarker and inter-study variations [3]. This variation encourages careful selection of the assays and limited biological interpretation.

Sirtuin-3 (SIRT3) is a member of the NAD⁺-dependent sirtuin family, which has a significant function in the deacetylation of mitochondrial proteins. It controls metabolic enzymes and pathways involved in stress response, which may impact mitochondrial function, respiratory metabolism, and redox homeostasis. SIRT3 is therefore a promising mechanistically interesting molecular target for metabolic disease research. Zhang et al. correlated the abnormalities of the granulosa cells' mitochondria and glucose metabolism with SIRT3-mediated effects, and Huang et al. studied mitochondrial sirtuins in women with PCOS [4, 5]. These studies offer a biologically relevant argument but do not equate circulating serum SIRT3 with intracellular SIRT3 activity. SIRT3 is thus suggested as a biochemical candidate marker and its use as a blood-based diagnostic biomarker requires appropriate analytical characterisation, rather than use as a blood-based diagnostic test.

Experimental studies of the role of SIRT3 in FOXO1/PGC-1 α signaling and cell responses to oxidative stress support the context in which SIRT3 is important [6]. SIRT3 also regulates multiple aspects of reactive oxygen species control at a more fundamental level by being reported to deacetylate and activate manganese superoxide dismutase (SOD2), a mitochondrial antioxidant enzyme [7]. The proposed antioxidant enzyme-SIRT3 pairing is interesting, but does not imply a correlation of serum SIRT3 with erythrocyte GPx among individuals. GPx is different in many respects to other antioxidant enzymes, utilizes peroxide substrates, has multiple isoforms, and can be measured in various matrices. Although a study of a low cost blood-based assay would help to establish a cause-and-effect relationship, such experiments that would measure relevant pathways and enzyme activity in the appropriate tissues are beyond the scope of this study.

Omentin-1, also known as intelectin-1, is an adipocyte secreted, circulatory protein studied in the context of metabolic regulation and insulin sensitivity. Tan et al. performed an important study in which they reported reduced circulating levels of omentin-1 in overweight insulin-resistant women with PCOS and investigated how insulin and glucose regulate omentin-1 [8]. Choi and colleagues noted lower circulating concentrations in women with PCOS who were not obese, signaling that it was not just obesity that was associated with this phenomenon [9]. Another meta-analysis confirmed the overall negative association between omentin-1 and PCOS in the included studies, but did not neglect the intra-study heterogeneity [10]. The results support the incorporation of omentin-1 in the model, but at the same time, BMI, adiposity distribution, glycemic state, medication, and the comparability of commercial immunoassays should be taken into consideration.

The evidence based in Iraq is relevant to the planned laboratory setting. Kadhim et al. [11] compared omentin-1 with other adipokines and biochemical parameters in Iraqi women who suffered from PCOS and/or PCOS associated infertility, and they noticed that omentin-1 levels were low in affected women. Kareem et al., also studied omentin-1, insulin resistance and metformin treatment in Iraqi PCOS patients, which further highlighted the importance of treatment history and metabolic phenotype when interpreting the circulating levels of omentin-1 [12]. The existence of these studies precludes a valid argument that the evaluation of omentin-1 in Iraqi women is novel itself. The potential value of the present design would be the detailed and specific biochemical comparison made between the groups as well as the effort to give an answer between group-level differences and within-group biochemical associations.

This is especially the case with medication related confounding. In certain women with PCOS, metformin is used and alters metabolic pathways and adipokines. Tan et al. noted changes in omentin-1 after metformin treatment, and stressed that treated and non-treated participants are not to be considered interchangeable biologically [13]. Plasma omentin-1 levels have also been associated with insulin resistance in non-obese PCOS women; the strength and independency of the associations vary according to population and adjustment method [14]. Therefore, a study measuring omentin-1 without the information needed on the use of medications, fasting insulin and glycemic status can only describe a circulating difference but is not well placed to explain the cause of that difference. This uncertainty can only be reduced by a limited amount due to age and BMI statistical adjustment.

Another proposed domain of a biochemical disturbance associated with PCOS is the low-grade inflammatory signaling. There has been evidence of immune-cell and cytokine dysregulation reported, but the syndrome is also heterogeneous; inflammatory findings do not necessarily indicate a single causal pathway, cautioned Banerjee et al. [15]. IL-1 β is a pro-inflammatory cytokine, which could be generated following inflammasome-dependent processing such as the NLRP3 pathway. Liu and co-workers have reported changes to inflammatory pathway markers in IL-1 β and mitochondrial oxidative stress in ovarian cell and follicular-fluid studies [16]. Ovarian follicular fluid and peripheral serum/plasma, however, are separate compartments. The absolute amounts of IL-1 β that are expected to be present in peripheral blood cannot be deduced from the amounts measured in follicular cells.

This is particularly evident in studies of the correlation between transcript, tissue protein and circulating levels of markers for PCOS, such as IL-1 β and inflammasome markers. Rostamtabar and colleagues analyzed the expression of genes and inflammatory pathways in women who have the syndrome [17]. More recently, Alenezi and co-workers reported a complex and often counterintuitive inflammasome composition in adipose tissue, and that a seemingly reasonable directional hypothesis could be incorrect when tested directly [18]. In addition, the concentration of IL-1 β in some samples may be close

to or outside the range of quantitation of traditional assays. Thus, using an assay with high sensitivity and validated for the intended use, including reporting below the lower limit of quantitation, is important. It would be problematic to remove below-range measurements from the data and replace them with arbitrary positive numbers or to interpret such data as representing actual concentrations of biological material.

The GPx variable is an appropriate ancillary measurement owing to its ability to give an output on the capacity of the circulating protein to reduce peroxides and hence provide a functional output of antioxidant activity rather than just protein abundance. Savic-Radojevic et al associated the hyperglycemia and hyperinsulinemia with glutathione peroxidase activity in women with PCOS [19]. An oxidative study among Omani women was conducted to investigate the behavior of antioxidant enzymes that included comparative support of the region [20]. For erythrocyte glutathione peroxidase, classic spectrophotometric methods are the demonstration of how enzyme activity can be measured using coupled biochemical reactions [21]. Caution is advised: Red-cell GPx activity (units/gram of hemoglobin) is a different analyte, compartment, and biological function than serum GPx protein concentration. Mechanical conclusions are not possible without an understanding of the different origins of such measurements when combined in a statistical model.

It was aimed to compare the levels of SIRT3 and omentin-1 in PCOS patients and control women, to describe the differences in IL-1 β and GPx activity and to evaluate the relationship between these biochemical parameters. The two additional analytes provided inflammatory and antioxidant context, with the most significant between-group differences being those for SIRT3 and omentin-1. Contrasts and ROC analyses were not planned but conducted as exploratory. The observational design does not allow for the analysis of inferences of biochemical mechanisms of causation or proof of diagnostic value.

2. Materials and Method

Hospital-based observational workflow

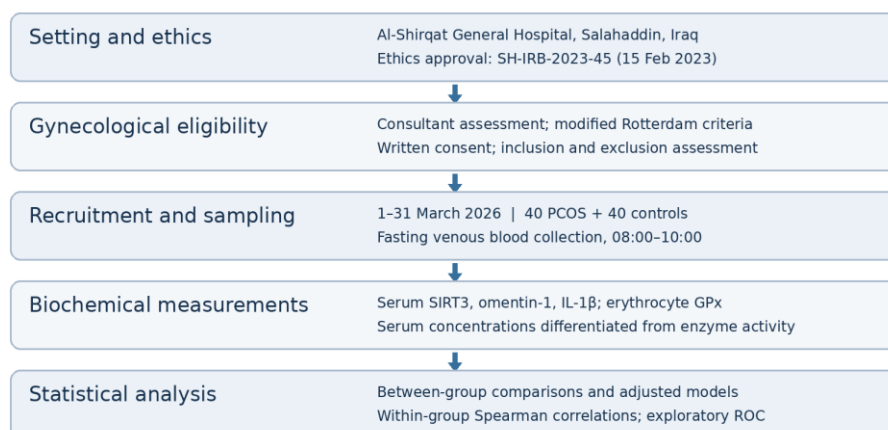


Figure 2. Observational study pathway and biochemical analysis: Al-Shirqat General Hospital, Salahaddin, Iraq, 1–31 March 2026.

2.1 Observational design, setting, and participants

This study was conducted in the Department of Obstetrics and Gynecology, and Clinical Laboratories, Al-Shirqat General Hospital, Al-Shirqat, Salahaddin, Iraq as a hospital based observational analytical case – control study. The recruitment and sampling period was between 1st March 2026 and 31st March 2026. There were 80 women included, 40 with PCOS and 40 controls. Consultant specialists performed a gynecological evaluation and informed written consent was obtained prior to participant enrollment.

Neither study intervention nor random assignment or therapy was added. The report was presented based on the STROBE recommendations for case–control studies [22].

A study identification code was assigned to each participant. Group status, age, BMI (weight/height²) and numerical laboratory observations of SIRT3, omentin-1, IL-1 β and GPx were analyzed. SIRT3, omentin-1, and IL-1 β were measured in the serum as concentration (ng/mL, pg/mL, respectively) while GPx was measured in the erythrocytes as enzymatic activity per g of Hb (U/g Hb). The groups of cases and controls were each assigned a code of 1 and 0, respectively, for modelling purposes. All laboratory values were used as is when evaluating observations.

2.2 Gynecological assessment, eligibility, and consent

The diagnosis of PCOS was based on the modified Rotterdam diagnostic criteria, which are stated in the international evidence-based PCOS guideline from 2023 [1]. All three of the following features, and none of the mimicking disorders, after exclusion were required for eligibility: oligo-ovulation or anovulation, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology by ultrasonography. The control women had regular menstrual cycles and no clinical hyperandrogenism. The diagnostic assessment was undertaken during recruitment in March 2026 following the publication of the international guideline in 2023.

The exclusion criteria were pregnancy, lactation, thyroid disease, hyperprolactinemia, congenital adrenal hyperplasia, exposure to hormonal contraceptive or metformin in the last three months and acute or chronic inflammatory disorders of the systemic organs. These limitations aimed to minimize alternative explanations for alterations in metabolic, immune and antioxidant indices. Participants were asked for consent prior to the blood collection and participant identifiers were not included in the analysis. Group characterization was based on age and BMI, and clinical eligibility was based on the treating gynecology team.

2.3 Biological specimens and pre-analytical documentation

The fasting venous blood was drawn between 08:00 and 10:00, with an average of 5 mL drawn from each subject. Specimens were separated into plain tubes for serum and EDTA-anticoagulated tubes for the preparation of erythrocytes. Serum was separated after clotting by centrifugation at 3000 $\times$ g for 10 minutes at 4 °C. Erythrocytes were separated, washed with phosphate-buffered saline and lysed for subsequent activity assessment. Aliquots of serum and red-cell lysate were stored at –80 °C and repeated freeze–thaw cycles were not experienced. In these procedures, separate analytical matrices were used for the soluble proteins and the enzyme activity of the erythrocytes.

A biochemical difference was preserved throughout the study. Circulating protein concentrations of SIRT3 and omentin-1, and an immunoassay-derived cytokine signal of IL-1 β , are reported; measurements of erythrocyte GPx are reported as an enzymatic rate (normalized to hemoglobin). The comparisons of the between-matrices data were not evaluated as evidence for a common intracellular process. Between-assay heterogeneity, which is well recognised in biomarker studies, and the quantification interval of the cytokine assay were also taken into account in the analytical discussion.

2.4 SIRT3 determination and analytical considerations

Human quantitative sandwich ELISA (MyBioSource, San Diego, CA, USA; catalog MBS163385; lot L230315582) was used to measure the immunoreactivity of the serum SIRT3. According to the manufacturer, this kit supports the measurement of a serum-compatible assay over the range of 0.1–40 ng/mL with an analytical sensitivity of 0.051 ng/mL. The absorbance of chromogenic microplate was measured at 450 nm with a microplate reader Multiskan GO (Thermo Fisher Scientific, USA). The results were measured as serum concentration of SIRT3 protein (ng/mL), rather than as catalytic activity

of SIRT3 in cells. So this measurement did not imply any mitochondrial deacetylase activity or expression in the ovarian tissue [4–7].

The measured levels of SIRT3 in serum were between 7.22 and 25.06 ng/mL. The biological basis for the study of this marker was based on mitochondrial protein acetylation, Reactive-Oxidation-Species defense and on the evidence of altered sirtuin pathways in models and patients with PCOS [4-6]. Comparisons of absolute values of circulating SIRT3 between studies should be done with analytical caution due to the influence of antibody recognition, matrix effects, calibration, and lot specifications. Do not interpret the concentration of serum antigen as an intracellular SIRT3 enzymatic activity.

2.5 Omentin-1 determination

Serum omentin-1 (intelectin-1, ITLN1) was quantified by human sandwich enzyme-linked immunosorbent assay (ELISA) using the reagents from Elabscience Biotechnology Inc. (Wuhan, China) and chromogenic microplate detection at 450 nm. All concentrations were reported as ng/mL. Omentin-1 was chosen as an adipokine associated metabolic variable due to previous reports of its association with adiposity, insulin resistance and PCOS status [8-14]. The study considered the assay result to be the circulating concentration of the antigen, as opposed to insulin sensitivity or functional activity of the adipose tissue.

The range of omentin-1 was found to be between 13.10 and 62.07 ng/mL. Absolute concentrations are dependent on the antibody specificity, calibration, dilution and on the values being within or outside the calibration range of the assay. Omentin-1 was thus regarded as a circulating biochemical marker and not as a surrogate marker of insulin sensitivity. The aforementioned studies have reported biochemical changes associated with PCOS and the effects of the metabolic phenotype and metformin exposure, respectively, and warrant cautious biochemical interpretation [8–14].

2.6 Measurement of IL-1 β

The levels of serum IL-1 β were evaluated using the R&D Systems Human IL-1 β /IL-1F2 Quantikine ELISA (Minneapolis, MN, USA; catalog DLB50) at 450 nm. Analytical sensitivity is reported to be ~1 pg/mL and the range of analytical measurement is 3.9–250 pg/mL in serum. Duplicate readings were taken following the laboratory procedures. Data which was below the range of the standard curve were considered as below quantification and were not entered as validated data for scientific inferences (group comparisons, Spearman association, ROC analysis) and hence were excluded from these analyses for IL-1 β .

Of the 80 recorded IL-1 β values, 78 (97.5%) were below 3.9 pg/mL. The two readings above this value were too limited to make a meaningful comparison between quantified IL-1 β concentration in the two clinical groups. IL-1 β was thus kept as a biologically relevant variable in the study rationale and the data highlighted the serum SIRT3-omentin-1 comparison and the erythrocyte GPx activity. If data fell outside the claimed method range, no replacement values were assigned to the data, the data were not arbitrarily imputed, nor were they presented as validated concentrations.

2.7 GPx enzymatic activity measurement

The activity of erythrocyte glutathione peroxidase (GPx) was determined by Glutathione Peroxidase Cellular Activity Assay Kit (Sigma-Aldrich, St. Louis, MO, USA; catalog CGP1). The coupled enzymatic method utilizes the oxidation of NADPH in the regeneration of reduced glutathione, while GPx-dependent peroxide reduction is used for the reduction of the peroxide. Under controlled assay conditions the decrease in absorbance at 340nm is a signal for the reaction rate [21]. Washing of the erythrocytes was done with phosphate buffered saline before hemolysis and measurement of hemoglobin was done by Drabkin's method. The activity of GPx was expressed as U/g Hb.

The range of GPx activity measured in the analysis was 17.80 - 50.00 U/g Hb. This indicator measured antioxidant enzymatic activity of the erythrocytes, not plasma GPx protein concentration. Not used as a direct measurement of ovarian oxidative damage, or mitochondrial superoxide dismutase activity. Absolute activity values may vary between studies due to variation in the age distribution of the erythrocytes and differences in the handling of samples, in the measurement of hemoglobin and in the calibration of the enzyme assays used for the previous redox studies [3,19–21].

2.8 Statistical analysis

The 80 complete records were used for analysis. Baseline variables and biomarkers were presented as means and standard deviations with distributional plots and medians if appropriate. Two-sided Welch unequal-variance *t* tests were performed to compare numbers between groups, and the mean difference and 95% CI were reported for PCOS minus control. Complementary rank-based assessments were provided by Mann–Whitney Tests. The adjustment for age and BMI has been performed using ordinary least-squares regression with HC3 heteroskedasticity-robust covariance, which is a sensitivity analysis instead of causal identification. The Benjamini–Hochberg false-discovery-rate procedure was used to assess multiplicity of the three major biochemical comparisons.

Spearman rank correlations were calculated for each group; PCOS and control. 78 of 80 IL-1 β values were outside the calibration range of the method and were not used to build the correlation interpretation. It was important to stratify by group as pooled correlations between markers may reflect case–control separation rather than any relationship within cases. Two-sided *P*-values and correlation values were reported, but without any causal interpretation. Exploratory analyses were performed with two-sided *P* < 0.05 being considered statistically significant.

ROCs were calculated for the orientation of each marker (lower values representing PCOS). Apparent AUC values were determined for each analyte. Standardized logistic regression was used to fit a model to each individual marker, and also a model of both markers (SIRT3–omentin-1), which were combined into a two-marker model. Out-of-fold predicted probabilities for each single marker model and the two-marker SIRT3–omentin-1 model were obtained by exploratory five-fold stratified cross-validation with *random_state* = 42 and pooled out-of-fold AUC were calculated. These estimates were considered to be descriptive and not externally validated diagnostic performance of the enrolled case–control sample.

2.9 Ethical approval and participant consent

The Institutional Ethics Committee of Al-Shirqat General Hospital reviewed and approved the study protocol on 15 February 2023 (approval reference SH-IRB-2023-45). All women gave informed consent prior to their participation. Participant identities were ensured by using coded study records. This study was observational and no changes in treatment or clinical management were made.

3. Results

3.1 Recruitment and baseline characteristics

Eighty participants were included, with 40 cases and 40 controls and complete numerical values for age, BMI, and four laboratory fields. Mean age was 26.16 ± 3.75 versus 27.00 ± 3.60 years (Welch *P* = 0.311), and mean BMI was 27.53 ± 2.57 versus 27.40 ± 2.94 kg/m² (*P* = 0.834). The resulting comparisons were consistent with broadly similar measured age and BMI distributions; nevertheless, nonsignificance does not establish covariate equivalence. Table 1 reports these characteristics.

Table 1. Baseline characteristics of women with PCOS and controls.

Variable	PCOS (n=40)	Controls (n=40)	P value
Age (years)	26.16 ± 3.75	27.00 ± 3.60	0.311
BMI (kg/m ²)	27.53 ± 2.57	27.40 ± 2.94	0.834

3.2 Serum SIRT3 and omentin-1 concentrations

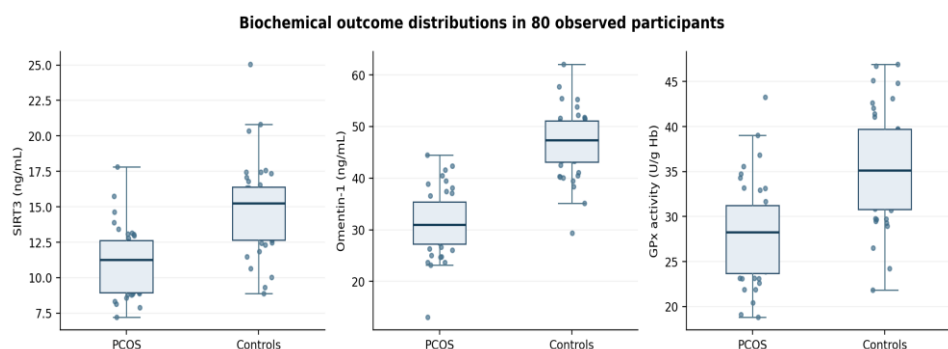
Mean serum SIRT3 was 11.17 ± 2.28 ng/mL in cases and 14.97 ± 3.15 ng/mL in controls (difference -3.80 ng/mL; 95% CI -5.02 to -2.57; Welch $P = 3.60 \times 10^{-8}$). The corresponding omentin-1 values were 31.41 ± 6.37 and 46.76 ± 6.40 ng/mL (difference -15.35; 95% CI -18.19 to -12.51; $P = 4.63 \times 10^{-17}$). Both contrasts were significant at $P < 0.001$. Distributional plots of the observed measurements appear in Figure 3.

3.3 Erythrocyte GPx activity and IL-1 β assay signals

Table 2. Biochemical group comparisons for SIRT3, omentin-1 and erythrocyte GPx activity. IL-1 β is reported only as the count below the lower quantitative boundary (3.9 pg/mL) of the specified DLB50 assay; a concentration mean or significance test was not calculated for this censored analyte.

Table 2. Comparison of SIRT3, Omentin-1, IL-1 β , and GPx Levels Between PCOS Patients and Controls

Marker	PCOS mean $\pm$ SD	Control mean $\pm$ SD	Mean difference (95% CI)	P value
SIRT3 (ng/mL)	11.17 ± 2.28	14.97 ± 3.15	-3.80 (-5.02 to -2.57)	3.60×10^{-8}
Omentin-1 (ng/mL)	31.41 ± 6.37	46.76 ± 6.40	-15.35 (-18.19 to -12.51)	4.63×10^{-17}
IL-1 β (pg/mL)	38/40 below 3.9 pg/mL	40/40 below 3.9 pg/mL	78/80 below quantifiable range	Not estimated
GPx (U/g Hb)	28.27 ± 5.50	35.41 ± 6.07	-7.14 (-9.72 to -4.56)	4.50×10^{-7}

**Figure 3.** Participant distributions of serum SIRT3, serum omentin-1 and erythrocyte GPx activity. Each point denotes one participant.

Erythrocyte GPx activity was 28.27 ± 5.50 U/g Hb among cases and 35.41 ± 6.07 U/g Hb among controls (difference -7.14 U/g Hb; 95% CI -9.72 to -4.56; Welch $P = 4.50 \times 10^{-7}$; Mann-Whitney $P = 1.50 \times 10^{-6}$). For IL-1 β , 38 of 40 case samples and all 40 control samples yielded numerical readings below the specified 3.9 pg/mL quantitative calibration boundary. Accordingly, between-group inference was restricted to SIRT3, omentin-1 and GPx activity, without treating sub-range cytokine values as validated concentrations. Table 2 presents these results, and Figure 3 displays individual observations for the three reportable biochemical outcomes.

3.4 Within-group Spearman correlations

Table 3. Within-group Spearman correlations for three interpretable biochemical measures.

Biomarker pair	PCOS (n=40)	Controls (n=40)
SIRT3–omentin-1	$\rho = -0.143$; $P = 0.380$	$\rho = -0.211$; $P = 0.190$
SIRT3–GPx activity	$\rho = -0.071$; $P = 0.662$	$\rho = -0.020$; $P = 0.904$
Omentin-1–GPx activity	$\rho = -0.055$; $P = 0.737$	$\rho = 0.181$; $P = 0.263$

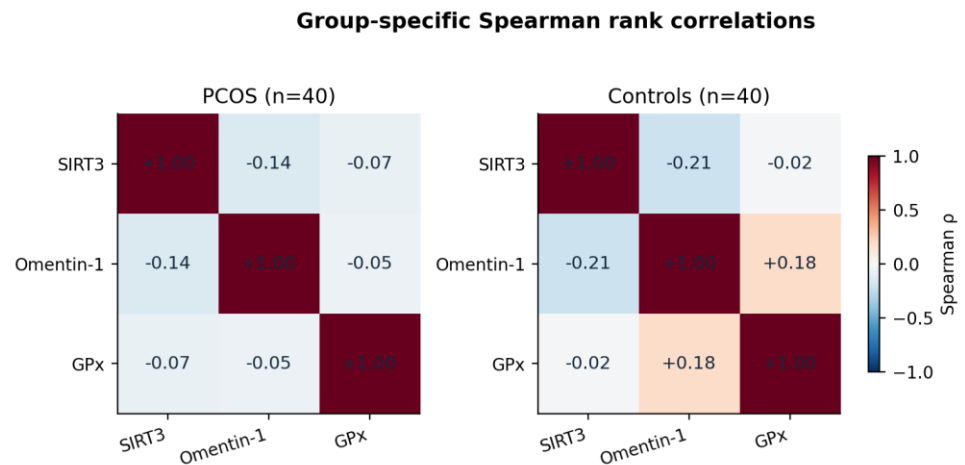


Figure 4. Heatmaps of Spearman correlations among SIRT3, omentin-1 and GPx activity, separately within cases and controls

Within the PCOS group, the three Spearman associations were nonsignificant: SIRT3–omentin-1, $\rho = -0.143$ ($P = 0.380$); SIRT3–GPx, $\rho = -0.071$ ($P = 0.662$); and omentin-1–GPx, $\rho = -0.055$ ($P = 0.737$). Within controls, the corresponding coefficients were -0.211 ($P = 0.190$), -0.020 ($P = 0.904$) and 0.181 ($P = 0.263$). Table 3 and Figure 4 display these group-stratified results. IL-1 β was excluded from inferential correlations because almost all measurements were outside the stated quantitative assay range.

This interplay of group level changes and statistically non-significant within group covariation is an important biochemical finding. Group averages are compared to find differences in biochemical distributions and Spearman correlation tests whether the individuals within the same clinical group are similarly ordered on pairs of biochemicals. The two questions are different. Caution is warranted that, in the absence of molecular associations within the biological population, apparently strong correlations can result from pooling cases with controls and the use of diagnostic strata. Those analyses in the present work are all performed within groups and do not suffer from that interpretive error, but nonsignificant coefficients cannot be considered biochemical independence.

3.5 Age- and BMI-adjusted group comparisons

Table 4. Age- and BMI-adjusted group coefficients for the three main biochemical indicators.

Outcome	Adjusted group coefficient	95% CI	P value
SIRT3 (ng/mL)	-3.87	-5.19 to -2.55	9.73e-09
Omentin-1 (ng/mL)	-15.51	-18.55 to -12.48	1.27e-23
GPx (U/g Hb)	-6.80	-9.48 to -4.11	7.07e-07

Age- and BMI-adjusted HC3-robust ordinary least-squares models indicated lower average values in the PCOS group: SIRT3 adjusted coefficient -3.87 ng/mL (95% CI -5.19 to -2.55; $P = 9.73\text{e-}09$), omentin-1 -15.51 ng/mL (95% CI -18.55 to -12.48; $P = 1.27\text{e-}23$), and GPx activity -6.80 U/g Hb (95% CI -9.48 to -4.11; $P = 7.07\text{e-}07$). These regressions adjusted only for the two recorded covariates, not other endocrine or metabolic characteristics.

3.6 Exploratory discrimination between selected groups

Table 5. Exploratory apparent and internally cross-validated AUC estimates for selected laboratory variables.

Marker or model	Apparent AUC	Five-fold CV AUC	Direction
SIRT3	0.846	0.832	lower in PCOS
Omentin-1	0.957	0.954	lower in PCOS
GPx	0.813	0.800	lower in PCOS
SIRT3 + Omentin-1	—	0.972	Combined model

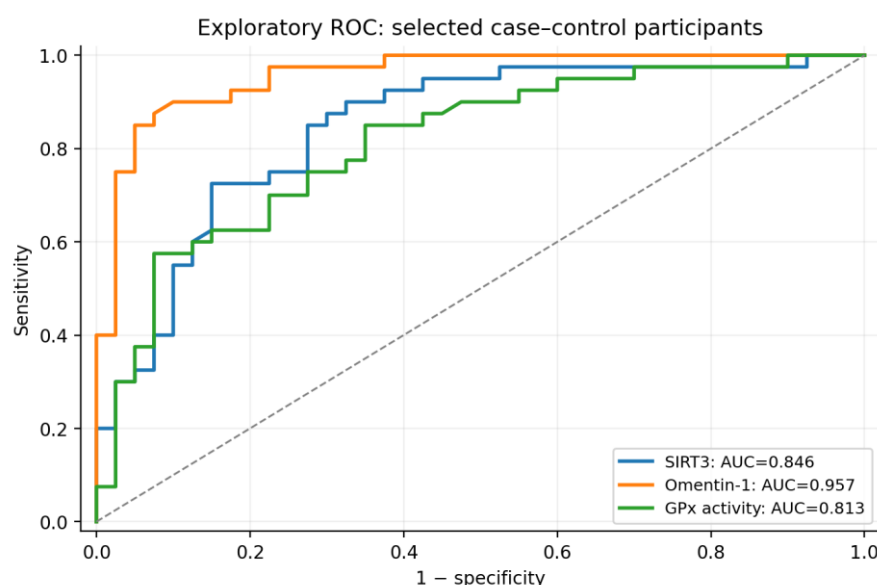


Figure 5. ROC curves for SIRT3, omentin-1 and GPx; AUCs are exploratory within the selected case-control sample.

Exploratory apparent ROC AUCs were 0.846 for SIRT3, 0.957 for omentin-1 and 0.813 for GPx activity. Using five-fold stratified cross-validated logistic-regression predictions (random seed 42) yielded pooled out-of-fold AUCs of 0.832, 0.954 and 0.800 for the individual markers, respectively, and 0.972 for SIRT3 plus omentin-1. These internally estimated AUCs describe numerical separation within selected case-control participants and do not establish population screening accuracy or clinical thresholds. Table 5 and Figure 5 summarize the discriminatory analyses.

The incidence of the case fraction was arbitrarily set at 40 per 80 patients and therefore cannot be extrapolated to the prevalence of PCOS within the community or an unselected clinic population. The calculated AUC in a case-control study is dependent on the contrasts and heterogeneity that are covered by the specifically chosen comparison groups. The separation may be less apparent if the same markers are used in the other endocrine disorders present in women. Before clinical recommendations are appropriate, a fully specified target population and reference standard and independent validation would be necessary.

4. Discussion

4.1 Interpretation of the overall biochemical profile

The primary results were the consistent differences in number between women with PCOS and controls in three biochemical parameters: circulating SIRT3 immunoreactivity, circulating omentin-1, and erythrocyte GPx activity. These differences remained in the age and BMI-adjusted models. However, there were no significant correlations within the PCOS group suggesting that parallel changes in group means need not be viewed as a molecular chain. Innovative methods for quantifying levels of the inflammatory marker IL-1 β based on the channel were also employed, but did not result in detectable recording signals. The overall results are consistent with biochemical heterogeneity, but not with any mechanism of linkage.

The power of the research is the ability to discriminate between contrasts in the observed groups and biochemical causality. Changing SIRT3 in the bloodstream may result from many tissues or cellular release patterns—not just from the mitochondria of the ovary. The level of omentin-1 could be an indicator of adipose-tissue endocrine activity along with metabolic exposures. The activity of GPx in the red cell plays a role in integrating the redox enzyme activity into the red-cell compartment. This is because these signals are from different pools of biological signals and a unidirectional mechanism is unclear. The lack of intra-group association also puts constraints on any argument implying a direct coordination of these processes between individuals.

4.2 SIRT3: metabolic regulation and mitochondrial context

SIRT3 is a mitochondrial sirtuin involved in the lysine deacetylation that requires NAD⁺ and involved in the coordination of enzymes involved in oxidative metabolism and stress responses. Previously, foundational research showed that SIRT3 plays a role in the regulation of manganese superoxide dismutase and mitochondrial ROS handling [7]. In cellular models relevant to PCOS, alterations of SIRT3 has been associated with alterations in energy metabolism and oxidative stress [4,6]. Therefore, the lower serum SIRT3 concentration seen in cases should be interpreted not as a decreased activity of SIRT3, but as a association with clinical PCOS status. A circulating immunoassay is unable to demonstrate tissue origin or catalytic function.

The difference in immunoreactivity and expression in cells is particularly significant. Studies are reported in different biochemical matrices, such as granulosa, peripheral blood mononuclear cells and follicular fluid [4,5]. The metabolic state of these compartments can be heterogeneous, as can the local tissue dynamics and effects of the phenotypes of PCOS. That is why two studies can observe opposite directions of SIRT3 change without contradicting each other if they are measuring changes in different biological compartments or in different lab readouts. An interpretation of biological material and assay principle is more convincing if the assay principle and the biological material are identified and only the measured analyte is concluded.

SIRT3 is also important for the concept of abundance vs. activity of proteins. A serum concentration assay is a test to determine the amount of antigen that is bound to antibodies when tested under the specific conditions of the reagent system. In contrast, further studies of SIRT3 activity involving deacetylation would need a different assay technique, typically an assay on isolated cells or tissues. The two measurements do not automatically imply local antioxidant protection or granulosa-cell bioenergetic capacity. In the present study serum SIRT3 level was thus used as a potential biochemical marker and not as a direct evaluation of mitochondrial reserve or ovarian cell viability.

4.3 Omentin-1 and metabolic biochemical heterogeneity

In the present study, cases had lower levels of serum omentin-1 in line with several prior studies of PCOS. Tan et al. reported that overweight insulin-resistant women with PCOS had decreased omentin-1 and investigated the regulation of omentin-1 by glucose

and insulin [8]. Plasma levels were reported to be lower in non-obese women [9] and a meta-analysis incorporated between trial variation [10]. The current case-control contrast is therefore biologically plausible and observational association alone cannot be used to determine the direction of the biologic effect, that is, whether alterations in omentin-1 are upstream or downstream from metabolic and reproductive changes.

An intra-regional comparison is possible from Iraqi studies. Kadhim and Hassan investigated the role of omentin-1 in Iraqi women with infertility due to PCOS [11], and Kareem et al studied serum levels of omentin-1 and its association to insulin resistance and metformin use [12]. They raise concerns about metabolic phenotype differences and selection and treatment histories for participants. Thus, the exclusion criterion for the present study was the use of recent metformin and hormonal therapy, criteria which differ from quantitative insulin resistance measures. Average BMI for groups does not mean average visceral adiposity, inflammatory state of adipose tissue or insulin action.

Omentin-1 is often described as an insulin sensitizing adipokine, however, the significance of lower levels of this molecule will depend on the tissue compartments that contribute to the circulation and how the physiological signal is controlled. Current results do not directly evaluate receptor mechanisms, Akt signaling, glycemic regulation or causal relationships of adipokine changes and ovarian physiology. Future biochemical studies should also incorporate fasting insulin and glucose or validated insulin-sensitivity markers, as well as add waist circumference and PCOS phenotypes. Such additions would allow for more specific hypotheses than a comparison of the status of disease.

4.4 IL-1 β and inflammation-related analytical interpretation

IL-1 β is an immune signaling cytokine linked to inflammasome activity and a variety of inflammatory events. Immune-cell activation and granulosa-cell microenvironments and NLRP3-related pathways have been studied in PCOS [15–18]. The valid circulating cytokine comparison requires an assay that will detect concentrations that are typical of healthy/typically inflamed populations, not only high cytokine concentrations. In the current laboratory observations, majority of the recorded IL-1 β values were below the published calibration range of the identified assay and, therefore, a definite serum IL-1 β group comparison was not recommended.

This is not a minor statistical point that simply because two distributions of optical-density noise are different does not mean that the same number of intensity digits assigned to the signals will create artificial group contrasts. The lower limit of detection and the lower limit of quantification also have different meanings: analytical detection is not always equal to reliable concentration determination. Thus, a high-sensitivity assay, validated for application of low-level serum specimens, is required prior to making a biological inference or ROC model using IL-1 β . However, this doesn't take away the fact that inflammation plays a role in PCOS, just that this specific cytokine channel can only set this up.

The paper highlights the importance of analytical chemistry in a strict clinical-biochemical manuscript and also gives an example of when to use it and when to avoid it. Immunoassays may differ with respect to amount of IL-1 β being measured, recognition of precursor molecules, calibration material, matrix effects, interference and background signal. A report that separates near-detection signals from those that are quantitatively validated will not lead to a false sense of certainty. In the study framework, the inflammatory arm is an important concept but the quantitative assessment of it in the blood is less conclusive than the other major observations of the sirtuin, adipokine system.

4.5 GPx activity and antioxidant defense

A decrease in the activity of GPx in erythrocytes indicates a correlation between disease state and one of the antioxidant defense pathways studied in erythrocytes. GPx can catalyze the reduction of peroxides through its interaction with glutathione which is

useful in protection from oxidative damage, and the activity can be determined by an enzyme coupled spectrophotometric assay [21]. The overall PCOS literature reports on abnormal circulating levels of oxidative-stress markers, which were reported in opposite directions and of different size in the various studies and analytes [3,19,20]. The present measurement is normalised to hemoglobin; as such, it describes erythrocyte activity, and is not reflective of serum GPx protein concentration.

It is indirect that the ovary is relevant to erythrocyte GPx. Antioxidant defense systems in mature erythrocytes are devoid of mitochondria, and the GPx system in this cell type is different than in granulosa cells, because of the different environment and biochemical processes in which each cell type exists. Furthermore, blood exposures are affected by the redox balance, erythrocyte turnover and sample processing. Thus, the results are consistent with a difference in an easily accessible peripheral antioxidant enzyme measurement, but not in direct measurement of oxidative stress within ovarian tissue. These compartments would be mechanistically connected by the use of assays suitable for the tissue or a more comprehensive systemic redox panel.

4.6 Interpreting within-group associations

Within the PCOS group, no association between pairwise Spearman relationship of SIRT3, omentin-1 and GPx activity was found statistically significantly. The three laboratory measures were not consistently coupled in the cases, although the group means varied very greatly. Therefore, it would be premature to draw a conclusion that a decrease in serum SIRT3 levels is an indicator of decreased red-cell GPx level in each patient. In contrast to the correlation analysis performed on pooled measurements of cases and controls, the present analysis reported associations within each group, which minimizes the likelihood that associations between analytes arise from the case status alone.

Biochemical independence does not mean there is no correlation. The study is not very powerful to detect moderate or non-linear relationships, since 40 participants are included in each stratum. In addition, some antigens are more abundant in serum than the others and the serum adipokine concentration and erythrocyte enzyme activity have varying biological regulation and time course. Correlations may be dampened by measurement variability and selection on a common clinical condition may change the observed covariance structure. The best possible inference is that the study failed to find any statistically significant within case monotonic associations for the sample size and analysis used.

4.7 Exploratory discrimination, not diagnostic certification

The three quantitative biochemical indicators had apparent AUCs in the range 0.81 – 0.96; the two-marker SIRT3–omentin-1 model had high internal cross-validated discrimination. These observations are not solely sufficient to make a claim of validated clinical biomarker. The contrast observed could be reduced by additional endocrine conditions and intermediate phenotypes in selected case–control groups, which might be more easily separated than women who presented prospectively with uncertain diagnoses. Thus, routine use of such a ROC plot would have no clinical sensitivity or specificity explicitly defined by either the current ROC plot or by internal resampling.

Prespecified intended use, assay traceability, data from a representative referral population, independent data, and benchmarking against accepted diagnostic standards would be required for definitive diagnostic validation [23]. Furthermore, the range of the assay, the analytical performance, and the lot-specific analytical validation and calibration should be valid for the specimens included in the assay before a laboratory decision threshold is meaningful. Since the research goal was not to develop a clinical diagnostic test, the output of the ROC is a secondary description of case–control separation. It is not a replacement for gynecological evaluation or the internationally recommended diagnostic criteria.

4.8 Context for clinical-biochemistry laboratories in Iraq

This study was conducted in an observational cohort of patients that were admitted to the hospital in Salahaddin, Iraq, and the markers chosen for their biochemical functions in metabolism, inflammation and oxidative balance. In Iraqi university and clinical laboratories, analytical principles which can be used are serum immunoassays and erythrocyte spectrophotometry. For distinct sample matrices, attention will be paid to the comparability; for within-group analysis, it will be avoided to mix up the separation of the diagnostic groups with a direct molecular relationship. These in concert, lend the research relevance to the discussion of feasible, non-invasive biochemical assessment locally.

Temporal heterogeneity is limited by a one-month recruitment window but not by itself by menstrual-cycle phase, subclinical infection, nutrition or seasonal factors. It is essential to determine the biological function of a serum protein, soluble cytokine, and red-cell enzyme, which is achieved by their different assays. Traces of detailed metabolic phenotyping along with matched assay traceability and the results of internal quality-control should be added to future replications. To evaluate the reproducibility of the observed differences in various hospitals and laboratory batches in Iraq, a multicenter design should be used.

The research into sirtuins and adipokines is ongoing, and now recent studies have drawn attention to the need for more than a statistically significant difference to be clinically relevant to define candidate biomarkers [2]. Biological basis for the exploration of redox measures [3] is supported by systematic syntheses of oxidative stress in PCOS, and inflammatory markers should be considered in a wider endocrine-metabolic context [15,16] are highlighted. One of the biochemical findings of this study is that it distinguishes the relationship of the abundance of analytes and enzymatic function rather than suggests that there is one causal cascade connecting the four variables.

4.9 Strengths and limitations

Some of the strengths of the study were that the women selected were clinically evaluated within a specific hospital and within 1 month, that the number of cases and controls was comparable, that written informed consent was obtained and that no additional measurements were constructed. The paper presents effect sizes and 95% confidence intervals, age/BMI adjusted models and Spearman associations by clinical status. It also preserves a biochemical separation between the amount of protein in the circulation and the activity of the enzymes within the cell. These options each help to avoid common problems in the interpretation of multiparameter observational biomarker studies.

Some limitations of this study were a single-center design, relatively small sample size, and limited analytic covariates (insulin, and PCOS phenotypes), as well as the absence of an independent validation cohort. The majority of the IL-1 β entries were below the claimed assay range of the assay. One control participant had age recorded as less than 18 years; the confirmation and consent procedures for this participant are relevant to assessment of the documentation of enrollment. Lot-specific assay characteristics and specimen traceability are still relevant for cross-study reproducibility, even though they are not vital, such as the correlation of the catalog identifier with the laboratory documentation at the time of the study.

4.10 Reproducibility and assay standards

Traceability from participant sample to analyte identity, to the calibration curve, to the linearity of the assay, to blank correction, to acceptance of the blank and quality-control is required for biochemical interpretation. The analytical plan used for the study separated the concentrations obtained by the immunoassay from GPx activity, and a good replication should also include lot identifiers, individual standard curves, in-run QC and hemoglobin normalization calculations. The latter is especially relevant for a low abundance cytokine

and a protein source that is not fully characterized in circulation, such as the protein in question. These conditions allow for reproducible evidence, not just for numerical precision.

There is no evidence to suggest that the metabolic, inflammatory and antioxidant domains are unrelated, but this is an empirical observation. Future cohorts can be used to investigate the hypothesized network by means of prespecified biochemical endpoints, insulin-resistance markers, biomarkers of oxidative-damage, and cell-specific SIRT3 readouts. The research should be expanded to include a wider range of case-control contrasts, as well as assay harmonization and external validation to identify results that are biologically consistent across contrasts.

5. Conclusion

In the hospital-based observational comparison of 40 women with PCOS and 40 controls, there were numerically lower serum levels of SIRT3 and omentin-1 in the PCOS group with highly significant calculated between-group contrasts, as well as lower (but not significantly lower) activity of GPx in the erythrocytes. In PCOS group, these three variables were not significantly correlated using Spearman correlation. IL-1 β was also of theoretical inflammatory relevance, but not measurable in this range of the assay in a serum-based manner. The observations suggest a stringent search for a biochemical pattern, which can be validated by assays, and does not imply molecular causation or performance of the assay as a diagnostic.

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