DOI: 10.24996/ijs.2026.67.5.4

ISSN: 0067-2904

Leucine-rich alpha-2-glycoprotein-1 as a Potential biomarker for polycystic ovary syndrome

Noor Jasim Mohamed, Ali Waleed Al-Ani*
Department of Chemistry, College of Science, University of Baghdad, Baghdad. Iraq

Received: 7/1/2025 Accepted: 17/6/2025 Published: xx

Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting women of reproductive age, characterized by anovulatory, infertility and metabolic disturbances. This study aimed to assess the diagnostic accuracy of Leucine-rich alpha-2-glycoprotein-1 (LRG1) and xanthine oxidase (XO) activity as novel biomarkers for PCOS, as well as to explore the relationship between LRG1 and XO activity across different groups. A total of 150 married women, aged 18-46 years were enrolled and divided into three groups: 50 with PCOS under treatment, 50 PCOS without treatment conditions shared by women with polycystic ovary conditions, and 50 healthy women. Serum samples were analysed to measure LRG1, xanthine oxidase (XO) activity, and various hormonal levels. The results demonstrated significantly elevated LRG1 levels and XO activity in PCOS patients, with a notable reduction in these markers among PCOS patients receiving metformin treatment compared to control. These findings suggest that LRG1 and XO activity may serve as reliable diagnostic biomarkers for PCOS. Additionally, correlations were observed between LRG1 levels, XO activity and hormonal parameters supporting their relevance in PCOS pathophysiology. This study highlights LRG1 and XO as promising diagnostic tools for PCOS management, warranting further investigation into their mechanistic roles and clinical applications

Keywords: diabetes millets**,** Leucine-rich alpha-2-glycoprotein-1, Luteinizing hormone, Percentage Body Fat**,** Polycystic ovary syndrome, Xanthine oxidase activity.

تقييم بروتين ألفا-2-جلايكوبروتين-1 الغني باللليوسين كعلامة حيوية محتملة لمتلازمة تكيس المبايض

نور جاسم محمد,علي وليد العاني*
الكيمياء, كلية العلوم, جامعة بغداد, بغداد, العراق

الخلاصة

متلازمة تكيس المبايض (PCOS) اضطراب غدد صماء شائع يصيب النساء في سن الإنجاب، ويتميز بانعدام الإباضة والعقم واضطرابات أيضية. هدفت هذه الدراسة إلى تقييم دقة تشخيص نشاط ألفا-2-جليكوبروتين-1 الغني باللليوسين (LRG1) وأوكسيديز الزانثين (XO) كمؤشرات حيوية جديدة لمتلازمة تكيس

*Email: ali.w@sc.uobaghdad.edu.iq

المبايض، بالإضافة إلى استكشاف العلاقة بين نشاط LRG1 و XOفي فئات مختلفة. تم تسجيل 150 امرأة متزوجة، تتراوح أعمارهن بين 18 و46 عامًا، وقُسِّمن إلى ثلاث مجموعات: 50 امرأة مصابة بمتلازمة تكيس المبايض ويخضعن للعلاج، و50 امرأة مصابة بمتلازمة تكيس المبايض دون علاج، ويشترك في ذلك نساء مصابات بحالات تكيس المبايض، و50 امرأة سليمة. حُللت عينات مصل الدم لقياس نشاط LRG1 وأوكسيديز الزانثين(XO) ، ومستويات هرمونية مختلفة. أظهرت النتائج ارتفاعًا ملحوظًا في مستويات LRG1 ونشاط XO لدى مريضات متلازمة تكيس المبايض، مع انخفاض ملحوظ في هذه العلامات لدى مريضات متلازمة تكيس المبايض اللواتي يتلقين علاجًا بالميتفورمين مقارنةً بالمجموعة الضابطة. تشير هذه النتائج إلى أن نشاط LRG1 و XO قد يكونان بمثابة مؤشرات حيوية تشخيصية موثوقة لمتلازمة تكيس المبايض. بالإضافة إلى ذلك، لوحظت ارتباطات بين مستويات LRG1 ونشاط XO والمعايير الهرمونية، مما يدعم أهميتها في الفيزيولوجيا المرضية لمتلازمة تكيس المبايض. تُسلط هذه الدراسة الضوء على LRG1 و XOكأدوات تشخيصية واعدة لإدارة متلازمة تكيس المبايض، مما يستدعي إجراء المزيد من البحث في أدوارهما الميكانيكية وتطبيقاتهما السريرية.

1. Introduction

Polycystic ovary syndrome (PCOS) is a significant and complex health condition affecting many women during their reproductive years [1]. It is a multifaceted endocrine and metabolic disorder and the leading cause of anovulatory infertility and excessive hair growth, and approximately 70% of affected women are still undiagnosed globally. In PCOS, ovulation is disrupted due to interrupted follicle development at an early stage, leading to the formation of multiple cysts when immature follicles develop over several cycles [2]. The diagnostic criteria for PCOS are varied and complex, creating a considerable risk of misdiagnosis when adolescents report menstrual issues or signs of increased androgen levels. For teenage diagnosis, fulfilment of at least four of five criteria is required. These criteria include primary or secondary amenorrhea, which can lead to irregular menstrual cycles, signs of clinical hyperandrogenism like severe hirsutism and ongoing acne, biological hyperandrogenism indicated by an LH/FSH ratio higher than two and plasma testosterone levels exceeding 50 ng/dl, as well as signs of glucose intolerance, such as acanthosis nigricans and abdominal obesity, suggesting hyperinsulinemia or insulin resistance, along with evidence of polycystic ovaries, PCOS is not merely a reproductive issue; it also affects skin health and mental well-being [3, 4]. The causes of this condition are multi-faceted, also the relationship between polycystic ovaries and proteins is multifaceted, involving hormonal imbalances, insulin resistance, and inflammatory processes. Understanding these relationships can help in developing targeted treatments for managing PCOS[5]. PCOS arises from a combination of genetic and environmental factors. Key hypotheses point to genetic influences that cause ovarian or neuroendocrine dysfunction, metabolic irregularities, and intricate interconnected mechanisms[6]. Alongside dysfunction of the hypothalamic-pituitary-ovarian axis, chronic inflammation and lifestyle factors – such as diet, stress, sleep issues, and environmental influences – may also contribute to the development of PCOS. Furthermore, PCOS is associated with various other health problems, including excess of abdominal fat such as corpulence, type 2 diabetes, and heart disease with also the increase in reactive oxygen species (ROS) and decreased of antioxidants capacity [7, 8]. Additionally, many women with PCOS suffer from anxiety and depression linked to concerns about their body image and fertility issues, complicating the condition further [9]. To effectively manage PCOS and reduce its associated risks, a thorough understanding of the disorder and commit to a tailored treatment plan.

Leucine-rich alpha-glycoprotein-1 (LRG1) is a glycoprotein that belongs to the leucine-rich repeat family, encoded by a protein-coding gene with eight leucine-rich repeats, at least four N-linked glycosylation sites, and two disulfide bonds. Researchers Haupt and Baudner first isolated LRG1 from human blood in 1977 [10]. The mature 50 kDa protein is released to the extracellular space and under normal conditions, is primarily produced by the liver and granulocytes [11]. LRG1 has been linked to the development of various diseases, including

inflammation, eye disorders, and certain cancers [12, 13]. It is involved in several biological processes, including signal transduction, cell differentiation, proliferation, migration, apoptosis, and cell cycle progression. It is considered a multifunctional protein with effects that vary depending on the context. LRG1's primary role is to regulate the TGF-beta signalling pathway, which can differ based on cell type and environment, this Dysregulation of TGF-beta signalling can lead to changes in ovarian tissue and reproductive issues, with systemic effects on other tissues potentially contributing to insulin resistance in women with PCOS[14].

Xanthine oxidase (XO) (EC:1.17.3.2) is an enzyme that catalyzes the conversion of hypoxanthine to xanthine and then to the final result uric acid , it is primarily found in the liver and intestines [15]. XO play a crucial role in purine metabolism and is also involved in the generation of reactive oxygen species which can contribute to oxidative stress that is associated with PCOS disease as well as in many diseases like diabetes, cardiovascular disease and metabolic syndrome [16]. To the best of our acknowledgement, no prior studies have explored this topic. Therefore, this study aimed to evaluate the potential use of LRG1 as a reliable diagnostic biomarker for different groups of married women with PCOS in combination with XO.

2- Methods

2.1 Study design

The present study included a total of 150 Iraqi married women aged 18-46 years, divided into three main groups:50 women with PCOS undergoing treatment with Metformin (Glucophage 500mg) + Primolut N (Norethisterone) 5mg during the period of sample collection,50 women with PCOS not receiving any drugs or supplements, and 50 healthy women, confirmed by physician examination based on criteria of Rotterdam ESHRE/ASRM 2003 which are: clinical feature, biochemical measurements, and ultrasound findings showing ovarian volume greater than 10 mL. Participants were recruited from private gynecology and Obstetrics clinical laboratories from the period August 2024 to November 2024. The protocol was approved by the Ethics Committee of the College of Science/ University of Baghdad. The consent of all patients was taken and they were informed that the samples were for research purposes. Ref.: CSEC/1124/0101. The participants groups of eligible 150 women were subjected to a list of several specific inquiries such as age, weight (kg), height(m), and anthropometric parameters such as Body mass index (BMI) were calculated by dividing weight (kg) by height (m^2), waist circumferences(cm), Hips circumferences(cm), waist to hips ratio(WHR) indicated fat distribution (WHR=waist/hips ratio), waist to height ratio(WHtR)(WHtR=waist/height ratio), marital status, menstrual and obstetrical and family history is it genetic, environmental or lifestyle habits, alongside fast food beside the type of drug they were taken. To ensure the most accurate results, certain medical conditions were excluded from this study. Women who were smokers, pregnant or recently given birth, had experienced a miscarriage, were of menopausal age, had fertility issues, or were taking medications or supplements other than those prescribed in the study were excluded. Cushing syndrome, rheumatoid arthritis or any type of inflammation, chronic diseases (such as diabetes mellitus, cardiovascular disease) or malignant tumours, or any diseases related to the ovary. A sample collection obtained by taken 10 mL of venous blood from each woman during the early follicular stage (2-3 day of menstrual cycle) for hormonal evaluation and for other related explorations through vacutainer and place it in gel tube for 20 min to coagulate after that centrifuge had undergone (centrifugation at 100.062 X*g* for 10 min) to obtain serum. The serum was separate and put it into an Eppendorf tube and stored at -20°C until the analyzing performed. Percentage Body Fat (PBF%) were measured by the InBody 270 device in Result Sheet displays body composition measurements by stand on the device and hold the hand electrodes. Direct Segmental Multi-Frequency BIA technology measures body segments

separately for an accurate analysis. Based on American College of sports Medicine (ACSM) and Agency for care Effectiveness (ACE) guideline for women, the healthy range is between 18-28%[17].

2.2 Biochemical Measurement

Biochemical assay

Serum Luteinizing hormones (LH), follicular-stimulating hormones (FSH), and testosterone levels were quantitatively determined using a sandwich chemiluminescence immune assay (ELISA) method performed by (cobas E411 analyser from Roche (04775279001), Germany), all results were calculated by the instrument using calibration curves which are stored by the tool. The LH expressed in mIU/mL, FSH in mIU/L (milli international Unit per liter), Hemoglobin A1c (HbA1c) value for human blood samples was determined using cobas c111 analyzer from Roche, Germany. Total serum protein and uric acid were measured by Linear Chemicals S.L. (Barcelona city, Spain) using spectrophotometers (PD-307, Japan) at wavelength 540 nm.

Determination of Leucine-rich alpha-2-glycoprotein-1

The concentration of LRG1 was assessed by FineTest Human LRG1 ELISA kit (catalogue No:EH2007) based on sandwich enzyme-linked immune-sorbent assay (China) Using HumaReader HS (HumanDiagnostics Worldwide, Germany) at wavelength= 450 nm using a standard curve.

Determination of xanthine oxidase activity

The enzymatic oxidation of XO was determined based on the Ackermann and Brill Methodology [18]. The specific activity was expressed as follows: XO sp. activity (U/g) = XO activity (U/L) / Total Protein conc. (g/L).

3. Statistical Analysis

Statistical analysis was performed using SPSS Statistics version 22 (2019) under a licensed agreement. The results were statistically analysed after conducting the normal distribution thus the data were presented as a Mean± Standard deviation (Mean± SD) and compared using Anova – LSD one-way. Pearson's correlation coefficient was used to determine the degree of association between continuous variables (r). Applying the Receiver Operational Characteristics curve (ROC) to assess the parameter accuracy for diagnosing PCOS

Results

The study comprised 150 Iraqi married women divided into three groups: a healthy/control group (n=50), PCOS patients without treatment (n=50), and PCOS patients receiving treatment (n=50). Demographic and characteristic data are presented in Table1. One-way ANOVA followed by LSD post hoc test was used to compare between three groups.

Table 1: Comparison of demographic and characteristics among studied groups

Parameters	Control group Mean ± SD (n=50)	PCOS without treatment Mean ± SD (n=50)	PCOS under treatment Mean ± SD (n=50)	*P-value*
Age (years)	32.68 ±9.52	30.68 ±6.59	29.96 ±9.52	0.324 0.583[a] 0.138[b] 0.358[c]
Weight (Kg)	73.40 ±12.03	75.12 ±17.05	77.20 ±17	0.930 0.147a 0.281b 0.336c
Height (cm)	161 ±3.53	159 ±6.41	159 ±4.27	0.357 0.227[a] 0.198[b] 0.191[c]
PBF%	39.09 ±4.47	39.52 ±7.78	39.14 ±7.52	0.846 0.220[a] 0.360[b] 0.410[c]
BMI(Kg/m^2)	47.19 ±7.75	47.48 ±9.96	45.08 ±5.46	0.152 0.221[a] 0.281[b] 0.364[c]
Waist(cm)	98.92 ±14.70	100.66 ±10.6	95.49 ±10.96	0.259 0.336[a] 0.347[b] 0.661[c]
Hips (cm)	107.40 ±14.39	109.98 ±11.6	108.08 ±13.8	0.523 0.120[a] 0.09[b] 0.336[c]
WHR	0.86 ±0.04	0.89 ±0.04	0.88 ±0.05	0.093 0.741[a] 0.799[b] 0.741[c]
WHtR	59.72 ±9.04	60.20 ±6.80	59.8 1±6.34	0.379 0.125[a] 0.139[b] 0.128[c]
LH(mIU/mL)	5.98 ±2.83	10.44 ±3.39	9.01 ±4.65	0.0001 0.0001[a] 0.004[b] 0.940[c]
FSH(mIU/mL)	7.19 ±2.06	6.07 ±2.45	6.52 ±1.87	0.004 0.002[a] 0.003[b] 0.308[c]
LH/FSH ratio	0.877 ±0.56	2.74 ±0.60	2.07 ±1.12	0.0001 0.001[a] 0.0001[b] 0.159[c]
HbA1C%	4.20 ±0.70	5.99 ±0.54	4.46 ±0.79	0.004 0.0001[a] 0.06[b] 0.002[c]
Total protein (g/L)	64.10 ±2.40	77.80 ±5.81	75.30 ±3.61	0.0001 0.0001[a] 0.0001[b] 0.0001[c]
Uric acid (mg/dL)	3.29 ±0.76	5.85 ±0.94	4.42 ±1.22	0.0001 0.0001 [a] 0.0001[b] 0.001[c]

The results are presented as mean ±SD. *P-value≤ 0.05* is considered as significant where (a) refer to comparison between healthy group and PCOS without treatment groups, (b) refer to comparison between healthy group and PCOS under treatment group, (c) refer to comparison between PCOS without treatment group and PCOS under treatment groups, Whereas: *=Significant (*P≤0.05*),**=Highly Significant *(P≤0.01),* PBF (percentage body fat), BMI (Body mass index), WHR (Waist to hips ratio), WHtR (Waist to height ratio), LH (luteinizing hormone), FSH (follicular-stimulating hormone), HbA1C (Hemoglobin A1C)

The table highlights that there were no significant differences in age, weight, height, waist, hips, WHR, WHtR PBF, and BMI among the studied groups (*P>0.05*). Similarly, levels of LH, FSH, LH/FSH ratio and total protein showed no significant differences between the untreated and treated PCOS groups (*P>0.05*). However, the level of LH, LH/FSH ratio, HbA1c, total protein and uric acid were significantly (*P<0.01*) elevated in both PCOS patients without treatment and those who were under treatment compared to healthy individuals.

This study aimed to investigate the differences in LRG1 level and XO activity, both of which were significantly elevated in PCOS patients without treatment compared to the control group *(P<0.01).* Nevertheless, the level of LRG1 was significantly dropped in PCOS patients who were under treatment compared to those patients who were untreated and healthy individuals *(P<0.01).* Although the XO activity was significantly decreased in PCOS patients under treatment compared to PCOS patients without treatment, this activity and the specific activity remained at a level higher than in the control group Table 2.

Table 2: Mean ±SD of LRG1 and XO activity and its specific activity of the studied group of married patients.

Parameters	Control group Mean ± SD (n=50)	PCOS without treatment Mean ± SD (n=50)	PCOS under treatment Mean ± SD (n=50)	P-*value*
LRG1 (ng/mL)	128.41±52.41	321.38 ±83.7	102.90 ±16.44	0.0001 0.0001[a] 0.001[b] 0.0001[c]
XO Activity (U/L)	35.58 ±2.61	70.84 ±7.02	48.36 ±5.12	0.0001 0.0001[a] 0.0001[b] 0.0001[c]
XO Sp.ac (U/g)	0.554 ±0.04	0.914 ±0.12	0.651 ±0.08	0.0001 0.0001[a] 0.05[b] 0.01[c]

The results are presented as mean ±SD. *P-value≤ 0.05* is considered as significant where (a) refer to comparison between healthy group and PCOS without treatment groups, (b) refer to comparison between healthy group and PCOS under treatment group, (c) refer to comparison between PCOS without treatment group and PCOS under treatment groups, Whereas: *=Significant (*P≤0.05*),**=Highly Significant (*P≤0.01*),Where as: LRG1(Leucin-rich α-2-glycoprotein-1), XO activity(Xanthine oxidase activity),XO Sp.ac (Xanthine oxidase specific activity)

The Pearson's correlations for LRG1 and XO activity with other studied parameters are shown in Table 3.

Table3: Pearson correlation for LRG1 and XO activity in the studied groups.

Parameters		PCOS without treatment		PCOS under treatment	
		LRG1 (ng/ml)	XO activity (U/L)	LRG1 (ng/ml)	XO activity (U/L)
LRG1 (ng/mL)	r.	1	0.011	1	0.611**
	P	-	0.850	-	0.0001
XO activity (U/L)	r.	0.011	1	0.611**	1
	P	0.850	-	0.0001	-
Total protein (g/L)	r.	0.706**	-0.026	0.064	-0.369
	P	0.0001	0.904	0.360	0.477
XO Sp.activity (U/g)	r.	-0.382	0.821**	0.912**	0.532
	P	0.060	0.0001	0.0001	0.006

Significant correlation at ($P \leq 0.05$)
****Significant correlation at ($P \leq 0.01$)****

In untreated PCOS patients, a significant negative correlation was observed between XO activity and BMI (r=-0.498, p=0.011). Conversely, a positive correlation was obtained between LRG1 and total protein (r=0.706, p=0.0001) and between XO activity and specific activity (r=0.821, p=0.0001). Among PCOS patients undergoing treatment, a positive correlation was obtained between LRG1 and each of XO activity (r=0.611, p=0.0001) and specific activity (r=0.532, p=0.006). Similarly, a strong positive correlation was noted between XO activity and its specific activity (r=0.912, p=0.0001). No other correlations were identified between the studied parameters with LRG1 or XO enzyme.

The receiver operating curve (ROC) was applied to examine the possibility of using LRG1 and XO enzymes as a marker for PCOS diagnosis Figure 1.

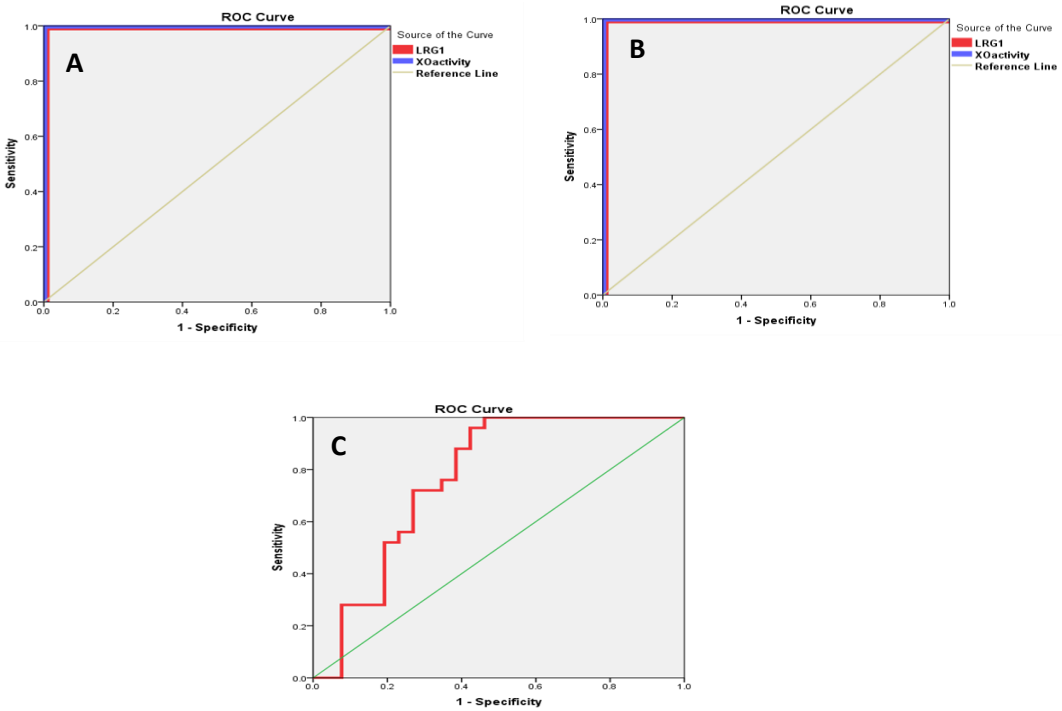

Figure1: ROC results (A): between control and PCOS patients without treatment (B): between PCOS patients without treatment and under treatment groups (C): LRG1 between Control and PCOS patients under treatment

According to ROC analysis, LRG1 displayed excellent discrimination between healthy individuals and PCOS patients without treatment as well as between PCOS patients without treatment and those who were under treatment (sensitivity 100%, specificity 100% and AUC 1). Similarly, XO activity demonstrated high sensitivity and specificity for discrimination between control and PCOS without treatment groups as well as between PCOS without treatment and PCOS under treatment (sensitivity 100%, specificity 100% and AUC 1), also LRG1 between control and PCOS under treatment estimate Sensitivity 88%, Specificity 62% and AUC 0.768) Table 4.

Table4: Receiver operating curve results for the three studied groups.

Parameters	Groups	AUC	Std Error	*p*-value	Cut-off value	Sensitivity	Specificity	CI(95%) Lower bound	Upper bound
LRG1 (ng/mL)	Control vs PCOS without treatment	1.000	0.0001	0.0001	258.57	100%	100%	1.000	1.000
	Control vs PCOS under treatment	0.768	0.070	0.001	122.76	88%	62%	0.631	0.904
	PCOS without treatment vs PCOS under treatment	1.000	0.0001	0.0001	259.12	100%	100%	1.000	1.000
XO activity (U/L)	Control vs PCOS without treatment	1.000	0.0001	0.0001	48.98	100%	100%	1.000	1.000
	PCOS without treatment vs PCOS under treatment	1.000	0.0001	0.0001	57.42	100%	100%	1.000	1.000

Discussion

The findings of this study underscore the complex nature of PCOS, emphasizing the critical roles of hormonal, metabolic, and inflammatory parameters in its pathophysiology. The study found no significant differences age, weight, height, PBF, BMI, WHR, WHtR among the groups. Regarding hormonal levels, LH exhibited elevation whereas FSH was dropped in both PCOS groups which agrees with other studies that suggest that elevated LH levels are a key hormonal disturbance in PCOS[19]. These levels are influenced by the gonado-hypothalamic-pituitary axis and can be affected by genetic, environmental, or obesity factors. Rapid pulsatility of gonadotropin-releasing hormone (GnRH) leads to increased LH to FSH levels, resulting in inadequate FSH for follicular development and higher LH stimulating ovarian androgen production, This imbalance also triggers negative feedback that inhibits FSH and LH secretion from the pituitary gland which in return they act on the ovaries [19, 20]. Furthermore, in patients receiving metformin treatment, a decrease in LH secretion and a slight increase in FSH levels were observed. These findings agree with a result of another study that attributed these changes to the effect of metformin drug on hormonal parameters [21]. The unbalance in LH/FSH ratio

indicates a significant state of luteinization without adequate follicle development. This is considered as one of the main causes of the decline in folliculogenesis and oogenesis in PCOS brought on by aberrant gonadotropin pulsatility and pituitary hypersecretion of LH which may contribute to anovulation and infertility issues, This state is associated with irregular menstrual cycle and clinical features of patients with PCOS[22]. It was also observed a significant improvement in the hormonal balances in PCOS patients under treatment. This is most likely a result of hyperactive gondatropes being inhibited and normalizing metabolic and hormonal status in various metabolic and endocrine disorders. This study also investigated HbA1c levels that were studied in other studies and gave similar results which reveal a significant increase in HbA1c levels in PCOS groups [23]. HbA1c provides evidence for the pre-diabetes risk, which contributes to metabolic complications such as type 2 diabetes and cardiovascular disease [24] and consequently leads to a significant rise in LRG1 levels among PCOS patients that was revealed in this study. The elevation in LRG1 occurs due to the existence of inflammatory conditions such as diabetes and cancers [25]. Since diabetes is an inflammatory condition and the severity of this inflammation is indicated by an increase in HbA1c levels a standard diagnosis marker for the presence of a high blood sugar [26]. A vital role of serum LRG1 was shown in the development of diabetic nephropathy through the association with the prognosis of diabetic nephropathy and cardiovascular illness [27]. In PCOS patients undergoing treatment, HbA1c levels significantly decreased, approaching those observed in the control group. This decrease was also observed in LRG1 after taking metformin which the later acts as a biguanide insulin sensitizer drug (a class of medications used to treat type 2 diabetes) by decreasing the amount of glucose production by the liver, increasing the absorption of glucose by the liver and skeletal muscle through insulin, and decrease the use of gluconeogenic substrates to enhance the sensitization of insulin in the body [28]. Another study suggests that metformin mechanism may be associated with the increase in insulin receptors' tyrosine kinase activity with the GLUT-4 glucose transporter recruitment besides the increase in the apoptosis process[29]. In rats, metformin found to be act by suppressing LRG1 and TGFβ1/ALK1-induced renal angiogenesis and confirmed the prevention of metformin on the ultrastructural alterations in rat diabetic nephropathy[30].It was also observed that total protein exhibits an increase in PCOS patients without treatment compared to control. A previous study showed a positive correlation between total protein and HbA1c in PCOS patients. It explained that the elevation of protein may result as a response to acute phase condition which is related to insulin resistance in PCOS patients suffering from pre-diabetes [31]. An elevation in total protein gives a signal for the body to respond to metabolic issues, highlighting the interconnectedness of inflammation and metabolic dysfunction. A positive correlation between LRG1 and total protein ($r=0.706$, $P<0.01$) may therefore explain the increase in LRG1 as a response to low-grade inflammation of PCOS which is associated with an increase of HbA1c and total protein. Furthermore, there was a significant rise in uric acid, XO activity, XO specific activity in PCOS patients and this agreed with reported studies [32]. Uric acid is the end product of the oxidation of xanthine by XO with the generation of reactive oxygen species (ROS). Reactive oxygen species are linked to many diseases as they have harmful effects through the irreversible damage to DNA as well as they oxidize and modify some cellular components and prevent them from performing their original functions[33-35] Thus the elevation in uric acid level may reflect the increase in XO activity associated with oxidative stress and metabolic disorders. Previous studies indicate that uric acid has paradoxical effects as an antioxidant by scavenging reactive oxygen species and as pro-oxidant [32]. The pro-oxidative properties of uric acid stimulate NADPH oxidases, leading to increased production of ROS which exacerbates insulin resistance, This diminishes the ability of the body to use insulin efficiently which leads to inflammation, dysfunctions in the vessel, pre-diabetes and consequently severe complications which is a main cause of elevating LRG1[36]. Another study indicates a relationship between

elevated uric acid and insulin resistance, a pre-diabetes state, in which high insulin may promote urate reabsorption in kidneys resulting in hyperuricemia[37]. In PCOS patients exposed to metformin show improved insulin sensitivity, and enhanced renal uric acid clearance thereby potentially reducing uric acid and managing gout effectively[38]. Furthermore, metformin is recognized for its role in reducing oxidative stress by reducing XO production via improving mitochondrial function, decreasing generation of ROS and reducing uric acid [39]. Additionally, it was observed that the elevation in XO activity was associated with an increase in LH/FSH ratio and fasting blood glucose the factors which correlated to PCOS incidence. Accordingly, this result suggests that the elevation in XO activity leads to the enhancement of oxidative stress and triggers an inflammatory response in PCOS which may stimulate the production of LRG1 as a part of the body's inflammatory response and oxidative damage. This is supported by a study which demonstrated the elevation in LRG1 is associated with oxidative stress in endothelial cells [40]. Regarding to PCOS patients taking metformin, it has been reported that metformin reduce the production of ROS by inhibit NADPH oxidase, generates cellular ROS to improve insulin action in indirect way to decreased oxidative stress damage and restore the balance of metabolic function [8]. This conclusion aligns with the findings of this study, which demonstrated a positive correlation between of LRG1 and XO activity and XO specific activity as well as a positive correlation between XO activity and XO specific activity in PCOS under metformin treatment. This suggests that XO enzyme is functioning efficiently in converting xanthine into uric acid, potentially contributing to oxidative stress in PCOS patients beside this elevation is associated with higher levels of inflammatory markers and metabolic risks factors.

In ROC curve, the result displays both LRG1 and XO activity demonstrated perfect sensitivity and specificity according to the area under curve (AUC) for distinguishing between healthy individual and those with PCOS in the untreated group and the treated group (100% sensitivity, 100% specificity for both LRG1 and XO activity). This highlights the potential utility of these biomarkers for early diagnosis and management of PCOS. The limitation of the study should measure at the genetic expression also no follow-up data to assess changes over time

Conclusion

This study reveals that LRG1 levels and XO activity are significantly elevated in women with PCOS. These markers are increased in untreated patients but decrease with metformin treatment, showing significant correlations with clinical parameters and underscoring their importance in the pathophysiology of PCOS. Additionally, both LRG1 and XO show excellent sensitivity and specificity for discrimination between healthy individuals and PCOS patients as well as between PCOS without and under treatment which introduce these parameters as potential biomarkers for diagnostic of PCOS. Further studies are required for supporting the current findings into LRG1's role in PCOS management.

Acknowledgements

Authors would like to acknowledge all patients with PCOS, all staffs of Gynecology and Obstetrics clinical and laboratories who participating in the study for their contribution.

Conflict of Interest

The authors declare that they have no conflicts of interest.

References

[1] S. Parua, M. P. Purkait, A. Bhattacharjee, R. Thangarajan, S. Rammohan, K. Islam, K. Bhattacharya, A. K. Syamal, "Exploring female infertility: A comprehensive review of polycystic ovary syndrome (PCOS) and its impact on reproductive health," *Obesity Medicine*, vol. 55, pp. 100619, 2025.

[2] Z. A. Alnisani and M .K. Abdulrida, "Effectiveness of Myoinositol alone or in Companion with Metformin in Improving Hormonal, Metabolic, and Clinical Features of PCOS Women," *Iraqi Journal of Pharmaceutical Sciences,* vol. 32, no. 1, pp. 61-73, 2023.

[3] J. P. Christ and M. I .Cedars, "Current guidelines for diagnosing PCOS," *Diagnostics,* vol. 13, no. 6, p. 1113, 2023.

[4] H. Almhmoud, L. Alatassi, M. Baddoura, J. Sandouk, M. Z. Alkayali, H. Najjar, and B. Zaino, "Polycystic ovary syndrome and its multidimensional impacts on women's mental health: A narrative review," *Medicine,* vol. 103, no. 25, p. e38647, 2024.

[5] S. Aggarwal and K. Pandey, "Early identification of PCOS with commonly known diseases: Obesity, diabetes, high blood pressure and heart disease using machine learning techniques," *Expert Systems with Applications,* vol. 217, p. 119532, 2023.

[6] H. Bai, H. Ding, M. J. C. Wang, E. Obstetrics, and Gynecology, "Polycystic Ovary Syndrome (PCOS): Symptoms, Causes, and Treatment," *Clinical and Expermental Obstetrics & Gynecology,* vol. 51, no. 5, p. 126, 2024.

[7] T. Jannink, E. Bordewijk, J. Aalberts, J. Hendriks, V. Lehmann, A. Hoek, M. Goddijn, M. van Wely. Reproduction, "Anxiety, depression, and body image among infertile women with and without polycystic ovary syndrome," *Human Reproduction,* vol. 39, no. 4, pp. 784-791, 2024.

[8] K. A. Adeshara, N. S. Bangar, P. R. Doshi, A. Diwan, and R. S. Tupe, "Action of metformin therapy against advanced glycation, oxidative stress and inflammation in type 2 diabetes patients: 3 months follow-up study," *Diabetes & Metabolic Syndrome Clinical Research & Reviews*, vol. 14, no. 5, pp. 1449–1458, 2020.

[9] M. Parker, A. Warren, S. Nair, and Barnard, "Adherence to treatment for polycystic ovarian syndrome: A systematic review," *PloS one,* vol. 15, no. 2, p. e0228586, 2020.

[10] G. De Rossi, M. E. Da Vitoria Lobo, J. Greenwood, and S. E .J. E. Moss, "LRG1 as a novel therapeutic target in eye disease," *Eye,* vol. 36, no. 2, pp. 328-340, 2022.

[11] D. Cai, C. Chen, Y. Su, Y. Tan, X. Lin, and R. Xing, "LRG1 in pancreatic cancer cells promotes inflammatory factor synthesis and the angiogenesis of HUVECs by activating VEGFR signaling," *Journal of Gastrointestinal Oncology,* vol. 13, no. 1, p. 400, 2022.

[12] C. Camilli, A. E. Hoeh, G. De Rossi, S. E. Moss, and J. Greenwood, "LRG1: an emerging player in disease pathogenesis," *Journal of Biomedical Science,* vol. 29, no. 1, p. 6, 2022.

[13] G. De Rossi, M. E. Da Vitoria Lobo, J. Greenwood, and S. E. Moss, "LRG1 as a novel therapeutic target in eye disease," *Eye,* vol. 36, no. 2, pp. 328-340, 2022.

[14] L. McIlvenna, "Polycystic Ovary Syndrome and Insulin Resistance: Dysregulation of Transforming Growth Factor Beta Signalling and the Effects of Exercise Training," Victoria University, 2021 .

[15] L. Liu, Y. Zhang, X. Wang, H. Meng, Y. He, X. Xu, H. Xu, C. Wei, X. Yan, and X. J. J. R .Tao, "Xanthine oxidase promotes hepatic lipid accumulation through high fat absorption by the small intestine," *Jounal of Hepatology Reports,* p. 101060, 2024.

[16] Furuhashi and Masoto , "New insights into purine metabolism in metabolic diseases: role of xanthine oxidoreductase activity," *American Journal of Physiology-Endocrinology and Metabolism* vol. 319, no. 5, pp. E827-E834, 2020.

[17] American College of Sports Medicine, "Physical Activity Guidelines - ACSM," ACSM, Feb. 17, 2025. https://acsm.org/education-resources/trending-topics-resources/physical-activity-guidelines/

[18] E. Ackermann and A. Brill, "Xanthine oxidase activity. in "methods of enzymatic analysis". Ed. Bergmeyer HU," ed: Academic Press, Inc., USA, 1974.

[19] T. G. Turki and J. W. Ammar, "Role of gonadotropin-releasing hormone, leptin hormone, luteinizing hormone, follicle-stimulating hormone, and obesity in polycystic ovarian syndrome," *International Journal of Scientific Research in Biological Sciences* vol. 11, no. 4, 2024.

[20] S. E. Ali, F. M. Khaleel, and F. E. Ali, "A study of apelin-36 and GST levels with their relationship to lipid and other biochemical parameters in the prediction of heart diseases in PCOS women patients," *Baghdad Science Journal,* vol. 17, no. 3 (Suppl.), pp. 924-930, 2020.

[21] Y. Guan, D. Wang, H. Bu, T. Zhao, and Hongwu, "The effect of metformin on polycystic ovary syndrome in overweight women: a systematic review and meta-analysis of randomized controlled trials," *International journal of endocrinology,* vol. 2020, no. 1, p. 5150684, 2020.

[22] M. Mitrašinović-Brulić, M. Buljan, and D. Suljević, "Association of LH/FSH ratio with menstrual cycle regularity and clinical features of patients with polycystic ovary syndrome," *Middle East Fertility Society Journal,* vol. 26, pp. 1-9, 2021.

[23] R. H. Melo, A. G. Pontes, L. Delmanto, F. N. Bueloni-Dias, H. D. L. Vespoli, and E. A. P. J. C. E. Nahas, "The role of glycated hemoglobin in the diagnosis of prediabetes and diabetes mellitus in young women with polycystic ovary syndrome," *Clinical Endocrinology,* vol. 100, no. 2, pp. 124-131, 2024.

[24] A. A. Rojekar, M. V. Rojekar, S. B. Ghanghurde and d. Lalla, "HbA1c in Polycystic Ovary Syndrome as the Cardiac Risk Assessment," *Iranian journal of diabetes and obesity,* vol. 14, no. 1, pp. 9-13, 2022.

[25] C. Camilli, A. E. Hoeh, G. De Rossi, S. E. Moss, and J. Greenwood, "LRG1: an emerging player in disease pathogenesis," *Journal of Biomedical Science*, vol. 29, no. 1, 2022.

[26] TN. Le, R. Bright, V-K. Truong, J. Li, R. Juneja, K.Vasilev, "Key biomarkers in type 2 diabetes patients: A systematic review, " *Diabetes, Obesity and Metabolism*, vol. 27, no. 1, pp. 7-22, 2025.

[27] C. Chen, J. Zhang, T .Yu, H. Feng, J. Liao, and Y. J. K. D. Jia, "LRG1 contributes to the pathogenesis of multiple kidney diseases: a comprehensive review," *kidney Dis (Basel),* vol. 1, no. 10.1159, p. 000538443, 2024.

[28] C. H. J. Choi, W. Barr, S. Zaman, C. Model, A. Park, M .Koenen, Z. Lin, S. K. Szwed, F. Marchildon, and A. J. E. Crane, "LRG1 is an adipokine that promotes insulin sensitivity and suppresses inflammation," *Elife,* vol. 11, p. e81559, 2022.

[29] M. Li, X. Chi, Y. Wang, S. Setrerrahmane, W. Xie, and H. Xu, "Trends in insulin resistance: insights into mechanisms and therapeutic strategy," *Signal transduction and targeted therapy,* vol. 7, no. 1, p. 216, 2022.

[30] H. M. Mohammad, S. G. Gouda, M. A. Eladl, A. Y. Elkazaz, K. S. Elbayoumi, N. E. Farag, A. Elshormilisy, B. B. Al-Ammash, A. Hegazy, S. M. J. B. Abdelkhalig, and Pharmacotherapy, "Metformin suppresses LRG1 and TGFβ1/ALK1-induced angiogenesis and protects against ultrastructural changes in rat diabetic nephropathy," *Biomedicine & Pharmacotherapy,* vol. 158 ,p. 114128, 2023.

[31] R.-X. Zeng, J.-P. Xu, Y.-Z. Zhang, J.-W. Tan, Y.-J. Kong, M.-Z. Zhang, and L.-H. J. F. i. E. Guo, "Associations of total protein, albumin, and globulin with insulin resistance: an NHANES study," *Frontiers in Endocrinology,* vol. 15 ,p. 1393137, 2024.

[32] Y.-N. Liu, H. Luo, X. Che, H. Peng, M. Li, and k. Liu, "Uric acid metabolism in polycystic ovary syndrome," *clinica Chimica Acta,* vol. 517, pp. 74-80, 2021.

[33] H. A. Hasan and A. W. Al-Ani, "Superoxide dismutase activity in breast cancer patients treated with anastrozole," *Onkologia i Radioterapia,* vol. 17, no. 11, pp. 001-010, 2023.

[34] A. W. Al-Ani, "Adenosine Deaminase and Guanine Deaminase: The Potential Role in Diabetic Foot Ulcers," *Iraqi Journal of Science* ,vol. 64, no. 10, pp. 4930-4941, 2023.

[35] N. M. Abd Al-Hameed and A. W. Al-Ani, "The role of monoamine oxidase and atherogenic index in newly diagnosed and tamoxifen treated women with breast cancer," *Journal of Medicinal and Chemical Science,* vol. 6 ,pp. 645-655, 2023.

[36] J. Shrestha, B. R. Pokhrel, M. Karki, N. Gautam, B. Tamang, G. K. Mandal, A. Palikhey, and B. Rayamajhi, "Comparative study of serum uric acid level in diabetic, pre-diabetic and non-diabetic individuals in a tertiary care center of Nepal," *Journal of chitwan Medical College,* vol. 12, no. 3, pp. 44-48, 2022.

[37] Y. Wu, H. He, K. Yu, M. Zhang, Z. An, H. Huang, "The association between serum uric acid levels and insulin resistance and secretion in prediabetes mellitus: a cross-sectional study," *Annals of Clinical & Laboratory science,* vol. 49, no. 2, pp. 218-223, 2019.

[38] Z. Gong, L. Zhang, and Y. Shi, "The potential role of uric acid in women with polycystic ovary syndrome ",*Gynecological Endocrinology* vol. 40, no. 1, p. 2323725, 2024.

[39] J. Feng, X. Wang, X. Ye, I. Ares, B. Lopez-Torres, M. Martínez, M.-R. Martinez-Larranaga, X. Wang, A. Anadón, "Mitochondria as an important target of metformin :The mechanism of action,

toxic and side effects, and new therapeutic applications," *Pharmacological research,* vol. 177, p. 106114, 2022.

[40] T. Miyazawa, N. Okami, N. Yukawa, S. Tsukamoto, S. Tanaka, K. Azushima, H. Wakui, and K. J. J. o. H. Tamura, "PS-B01-5: TISSUE-SPECIFIC REGULATION OF LRG1 EXPRESSION IN MULTIPLE HYPERTENSION MODELS OF MOUSE," *Journal of Hypertension,* vol. 41, no. Suppl 1, pp. e171-e172, 2023.