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Plasma and Erythrocyte Prolidase Enzyme Kinetics as Functional Biomarkers of Extracellular Matrix Remodeling in Polycystic Ovary Syndrome

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ABSTRACT: Background: Polycystic ovary syndrome (PCOS) is a complex endocrine and metabolic disorder characterized by hyperandrogenism, chronic anovulation, insulin resistance, and reproductive dysfunction. Increasing evidence suggests that extracellular matrix (ECM) remodeling and ovarian fibrosis are integral components of PCOS pathophysiology. Prolidase (peptidase D; PEPD), the key enzyme responsible for the final step of collagen degradation, plays a pivotal role in collagen turnover and ECM homeostasis. Although altered prolidase concentrations have been reported in women with PCOS, little is known about the functional kinetic behavior of the enzyme in different biological compartments.

Objective: This study aimed to evaluate plasma and erythrocyte prolidase enzyme kinetics in women with PCOS and to determine whether alterations in Michaelis–Menten kinetic parameters could serve as functional biomarkers of extracellular matrix remodeling and ovarian fibrosis.

Methods: A case–control study was conducted involving 120 women aged 18–40 years, including 60 women diagnosed with PCOS according to the updated Rotterdam ESHRE/ASRM criteria and 60 age- and body mass index-matched healthy controls. Plasma and erythrocyte hemolysates were prepared from fasting venous blood samples. Prolidase activity was determined spectrophotometrically using glycyl-L-proline as the substrate at different substrate concentrations. Michaelis–Menten and Lineweaver–Burk analyses were performed to determine the Michaelis constant (K_m) and maximum reaction velocity (V_{max}) in plasma and erythrocyte compartments. Hormonal, metabolic, and lipid parameters were evaluated using standard laboratory methods to characterize the clinical phenotype of the study population.

Results: Women with PCOS demonstrated marked alterations in prolidase enzyme kinetics compared with healthy controls. Plasma prolidase exhibited a lower K_m value (25.64 vs. 28.57 mM), indicating increased substrate affinity, accompanied by a marked reduction in V_{max} (16.54 vs. 30.62 $\mu\text{mol proline min}^{-1} \text{mg}^{-1}$), suggesting impaired

catalytic capacity. In contrast, erythrocyte prolidase showed an increased K_m (38.46 vs. 29.41 mM) together with a reduced V_{max} (46.96 vs. 58.01 $\mu\text{mol proline min}^{-1} \text{mg}^{-1}$), reflecting diminished substrate affinity and catalytic efficiency. These compartment-specific kinetic alterations indicate that prolidase dysfunction in PCOS extends beyond changes in enzyme concentration and involves fundamental modifications in enzyme behavior.

Conclusions: The present findings demonstrate that women with PCOS exhibit significant functional alterations in prolidase enzyme kinetics in both plasma and erythrocyte compartments. These kinetic abnormalities may reflect disturbed collagen turnover, extracellular matrix remodeling, and progressive ovarian fibrosis. Assessment of prolidase kinetic parameters provides functional information beyond conventional enzyme measurements and may represent a promising biomarker for evaluating extracellular matrix dysregulation in women with PCOS.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common endocrine and metabolic disorder affecting women of reproductive age and represents the leading cause of chronic anovulation and infertility worldwide. Depending on the diagnostic criteria and the studied population, its prevalence ranges from 6% to 20%, making it a major public health concern with significant reproductive, metabolic, and psychological consequences [1–3]. In addition to menstrual irregularities and hyperandrogenism, women with PCOS frequently exhibit insulin resistance, obesity, dyslipidemia, impaired glucose metabolism, and an increased risk of developing type 2 diabetes mellitus and cardiovascular disease [2–5].

Although PCOS has traditionally been considered an endocrine disorder, accumulating evidence indicates that structural alterations within the ovarian microenvironment play an equally important role in disease progression. Recent histopathological and molecular studies have demonstrated excessive collagen deposition, stromal expansion, thickening of the tunica albuginea, and increased ovarian stiffness, all of which are characteristic features of progressive ovarian fibrosis and abnormal extracellular matrix (ECM) remodeling [6–9]. These structural abnormalities disrupt follicular development by altering the communication between granulosa cells, theca cells, and the surrounding extracellular matrix, thereby contributing to follicular arrest and chronic anovulation [8–10].

The extracellular matrix is a highly dynamic three-dimensional network composed primarily of collagens, laminins, fibronectin, elastin, and proteoglycans. Beyond its structural role, the ECM regulates cell adhesion, migration, proliferation, differentiation, angiogenesis, and intracellular signaling pathways that are essential for normal folliculogenesis and ovulation [9–11]. Physiological ovarian remodeling depends on a tightly regulated balance between collagen synthesis and degradation. Disruption of this balance promotes excessive extracellular matrix accumulation, resulting in progressive fibrosis and impaired ovarian function [7,10].

Collagen turnover is primarily regulated through enzymatic degradation, in which prolidase (peptidase D, PEPD) plays a fundamental role. Prolidase is a manganese-dependent cytosolic exopeptidase that catalyzes the final step of collagen degradation by hydrolyzing imidodipeptides containing C-terminal proline or hydroxyproline residues. Through recycling of these amino acids, prolidase provides substrates required for collagen resynthesis, tissue repair, and maintenance of extracellular matrix homeostasis [12–15]. Consequently, alterations in prolidase function may directly influence collagen metabolism and contribute to pathological tissue remodeling in several fibrotic and metabolic disorders [14–17].

Most previous studies investigating prolidase in PCOS have focused primarily on measuring circulating enzyme concentration or total enzymatic activity. Although these measurements provide valuable biochemical information, they do not adequately reflect the functional characteristics of the enzyme. Enzyme kinetic analysis offers a more comprehensive assessment of enzyme behavior by evaluating substrate affinity and catalytic efficiency through the determination of the Michaelis constant (K_m) and maximum reaction velocity (V_{max}). Changes in these parameters may indicate alterations in enzyme conformation, substrate binding, post-translational modifications, or intracellular regulatory mechanisms that cannot be detected by concentration measurements alone [16–19].

In addition, increasing evidence suggests that chronic hyperinsulinemia, oxidative stress, and persistent low-grade inflammation associated with PCOS may modify enzyme structure and catalytic function, thereby affecting collagen turnover and extracellular matrix remodeling [5,17,20]. Because erythrocytes and plasma represent biologically distinct compartments with different metabolic environments, comparative evaluation of prolidase kinetics in both compartments may provide valuable insight into systemic and cellular alterations associated with PCOS [18–21].

Despite growing recognition of extracellular matrix remodeling in PCOS, studies evaluating prolidase enzyme kinetics remain extremely limited, and little is known regarding the functional behavior of prolidase in plasma and erythrocyte hemolysates. Therefore, investigating kinetic parameters rather than enzyme concentration alone may provide novel mechanistic insights into collagen metabolism and ovarian fibrosis.

Accordingly, the present study aimed to characterize prolidase enzyme kinetics in plasma and erythrocyte hemolysates of women with PCOS using Michaelis–Menten and Lineweaver–Burk kinetic models. Furthermore, the study sought to determine whether alterations in K_m and V_{max} values could serve as functional biomarkers of extracellular matrix remodeling and provide additional insight into the pathophysiological mechanisms underlying ovarian fibrosis in women with PCOS.

PATIENTS AND METHODS

This case–control study was conducted at the Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq, between April and October 2025. A total of 120 women aged 18–40 years were enrolled, including 60 women diagnosed with PCOS according to the updated Rotterdam ESHRE/ASRM criteria and 60 age- and body mass index-matched healthy controls. Women with thyroid disorders, hyperprolactinemia, congenital adrenal hyperplasia, Cushing's syndrome, diabetes mellitus, hepatic or renal diseases, inflammatory disorders, malignancy, pregnancy, or hormonal treatment within the preceding three months were excluded. The study protocol was approved by the Scientific and Ethical Committee of the College of Medicine, Al-Nahrain University, and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki [22,23].

Following an overnight fast of 10–12 h, approximately 10 mL of venous blood was collected during the early follicular phase of the menstrual cycle. Plasma, serum, and erythrocyte hemolysates were prepared using standard laboratory procedures. Prolidase activity was determined spectrophotometrically using glycyl-L-proline as the substrate according to Myara et al. with minor modifications [24]. Enzyme activity was measured at different substrate concentrations, and Michaelis–Menten and Lineweaver–Burk analyses were performed to determine the Michaelis constant (K_m) and maximum reaction velocity (V_{max}). Catalytic efficiency was estimated using the V_{max}/K_m ratio.

Fasting blood glucose, serum insulin, and lipid profile were measured using standard laboratory methods. Insulin resistance was calculated using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) according to Matthews et al. [25]. Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), anti-Müllerian hormone (AMH), testosterone, estradiol, progesterone, prolactin, and thyroid-stimulating hormone (TSH) were determined by electrochemiluminescence immunoassay (ECLIA) according to the manufacturers' instructions. Statistical analyses were performed using IBM SPSS Statistics version 26.0. Continuous variables were expressed as mean $\pm$ standard deviation (SD), and comparisons between groups were performed using the independent Student's *t*-test. Statistical significance was defined as $P < 0.05$.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software (Version 26.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), while categorical variables were expressed as frequencies and percentages.

Differences between women with PCOS and healthy controls were evaluated using the independent Student's *t*-test. Enzyme kinetic parameters were compared descriptively between groups, and catalytic efficiency was calculated from the V_{max}/K_m ratio. Statistical significance was considered at $P < 0.05$.

RESULTS

Characteristics of the Study Population

A total of 120 women were enrolled in the present study, comprising 60 women diagnosed with PCOS and 60 apparently healthy controls. The two groups were comparable regarding age and body mass index (BMI), with no statistically significant differences observed. This matching minimized the potential influence of demographic variables on prolidase kinetic analysis.

Table 1. Demographic characteristics of the study population

Variable	PCOS (n=60)	Controls (n=60)	P value
Age (years)	27.08 ± 4.49	25.87 ± 4.17	0.127
BMI (kg/m ²)	28.50 ± 3.31	27.62 ± 2.53	0.107
Disease duration (years)	4.97 ± 2.63	—	—

Hormonal and Metabolic Characteristics

Women with PCOS demonstrated the characteristic endocrine and metabolic abnormalities of the syndrome. Serum LH, testosterone, AMH, prolactin, estradiol, fasting glucose, insulin, HOMA-IR, total cholesterol, triglycerides, and LDL-cholesterol were significantly higher than those of healthy controls, whereas progesterone concentrations were significantly lower.

Table 2. Hormonal and metabolic characteristics of women with PCOS and controls

Variables	PCOS patients (n=60)	Controls (n=60)	p- value
FSH, mIU/ml			
Mean ±SD	6.13±1.32	6.0±1.53	0.586
Range	3.51- 9.45	3.24-9.95	
LH, mIU/ml			
Mean ±SD	11.66±4.0	6.2±2.23	<0.001
Range	1.4-19.02	0.61-11.15	
Testosterone, ng/dl			
Mean ±SD	75.62±13.62	40.33±10.2	<0.001
Range	42.71-112.4	20.06-65.8	
AMH, ng/ml			
Mean ±SD	7.0±1.92	2.73±0.9	<0.001
Range	2.06-11.54	1.2-4.91	
Progesterone, ng/ml			
Mean ±SD	5.34±2.11	9.86±2.58	<0.001
Range	1.02-9.57	4.2-15.53	
Estradiol, pg/ml			
Mean ±SD	61.89±18.61	54.73±14.15	0.019
Range	16.02-103.27	27.26-87.9	
Prolactin			

Mean $\pm$ SD	15.33 $\pm$ 5.14	12.38 $\pm$ 3.96	0.001
Range	0.76-25.71	2.3-20.3	
TSH, μIU/ml			
Mean $\pm$ SD	2.26 $\pm$ 1.02	2.25 $\pm$ 0.88	0.867
Range	0.05-4.5	0.3-3.92	
LH/FSH ratio			
Mean $\pm$ SD	2.0 $\pm$ 0.83	1.09 $\pm$ 0.46	<0.001
Range	0.24-4.66	0.1-2.28	

Diabetes-Related Parameters

Table 3 compares diabetes-related parameters between PCOS patients and controls. Fasting blood sugar was significantly higher in PCOS patients than in controls (95.45 $\pm$ 9.14 vs 89.82 $\pm$ 10.44 mg/dl, $p = 0.002$). Serum insulin levels were also markedly elevated in the PCOS group (16.0 $\pm$ 6.83 vs 8.38 $\pm$ 3.0 μ IU/ml, $p < 0.001$). Likewise, HOMA-IR was significantly higher among PCOS patients (3.73 $\pm$ 1.6 vs 1.86 $\pm$ 0.69, $p < 0.001$).

Regarding the frequency of insulin resistance, 50 PCOS patients (83.33%) had insulin resistance compared with only 7 controls (11.67%) with a highly significant difference ($p < 0.001$).

Table 3. Diabetes-Related Parameters in patients and controls

Variables	PCOS patients (n=60)	Controls (n=60)	p- value
FBS, mg/dl			
Mean $\pm$ SD	95.45 $\pm$ 9.14	89.82 $\pm$ 10.44	0.002
Range	77.62-120.11	57.6-111.3	
Insulin, μIU/ml			
Mean $\pm$ SD	16.0 $\pm$ 6.83	8.38 $\pm$ 3.0	<0.001
Range	2.79	1.63-14.57	
HOMA-IR			
Mean $\pm$ SD	3.73 $\pm$ 1.6	1.86 $\pm$ 0.69	<0.001
Range	0.61-8.42	0.23-3.4	
Insulin resistance			
No	10(16.67%)	53(88.33%)	<0.001
Yes	50(83.33%)	7(11.67%)	

Lipid Profile

Total cholesterol was significantly higher in PCOS patients than in controls (207.57 $\pm$ 29.0 vs 176.55 $\pm$ 25.55 mg/dl, $p = 0.021$). Triglyceride levels were also significantly elevated in the PCOS group (150 $\pm$ 45.77 vs 119.45 $\pm$ 36.45 mg/dl, $p = 0.019$). Likewise,

LDL cholesterol was significantly higher among PCOS patients compared with controls (124.65 ± 24.74 vs 107.47 ± 17.36 mg/dl, $p=0.033$) as shown in table 4.

Table 4. Lipid profile in patients and controls

Variables	PCOS patients (n=60)	Controls (n=60)	p- value
TC, mg/dl			
Mean $\pm$ SD	207.57 ± 29.0	176.55 ± 25.55	0.021
Range	141-264.9	115.22-225.65	
TG, mg/dl			
Mean $\pm$ SD	150 ± 45.77	119.45 ± 36.45	0.019
Range	20.75-234.81	48.37-201.06	
HDL, mg/dl			
Mean $\pm$ SD	50.31 ± 9.53	53.74 ± 10.77	0.067
Range	26.78-73.9	25.79-86.93	
LDL, mg/dl			
Mean $\pm$ SD	124.65 ± 24.74	107.47 ± 17.36	0.033
Range	57.42-179.27	61.55-143.78	

Kinetic studies for determination the prolidase activity in plasma and erythrocyte hemolysate

Kinetic studies were used for determination the activity of prolidase and to obtain the Michaelis constant (k_m) and maximum velocity (V_{max}) in plasma and erythrocyte hemolysate of PCOS patients compared with control using spectrophotometric method as shown in the figure (1), (2) and kinetics enzyme prolidase in plasma and erythrocyte hemolysate by – Line weaver-Burk plot as shown in the figure (3), (4)

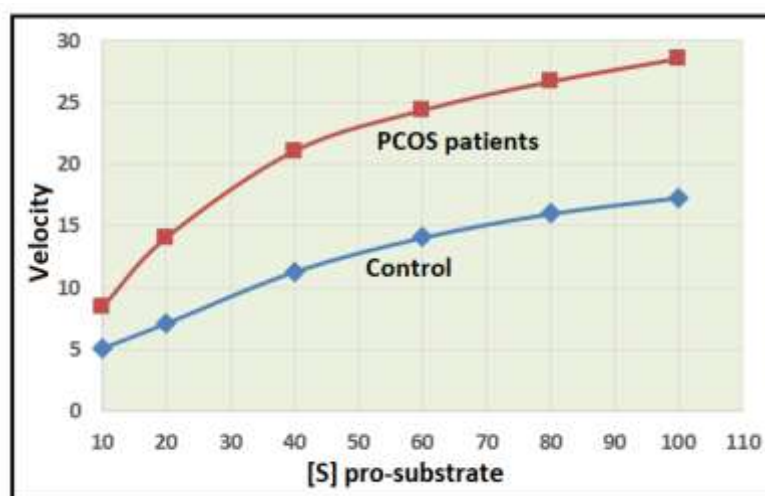


Figure 1: Kinetics of prolidase in plasma of PCOS patients and controls. Michaelis Menten equation

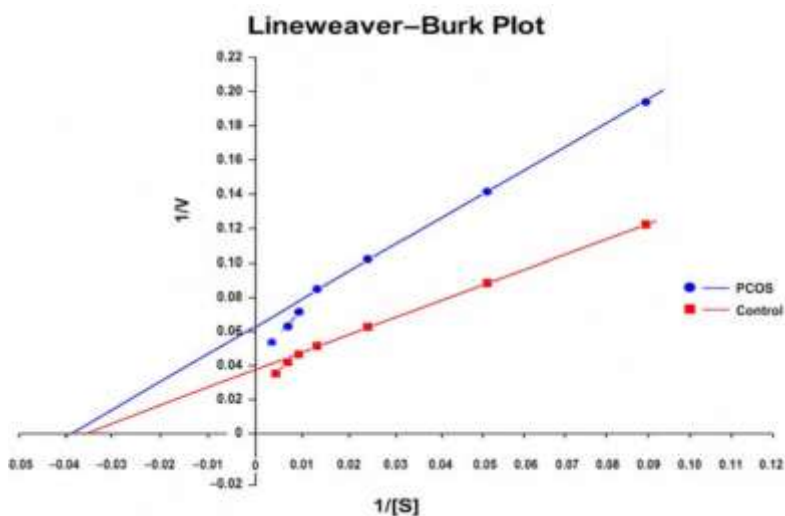


Figure 2: Kinetics of prolidase in plasma of PCOS patients and controls. Line weaver-Burk plot

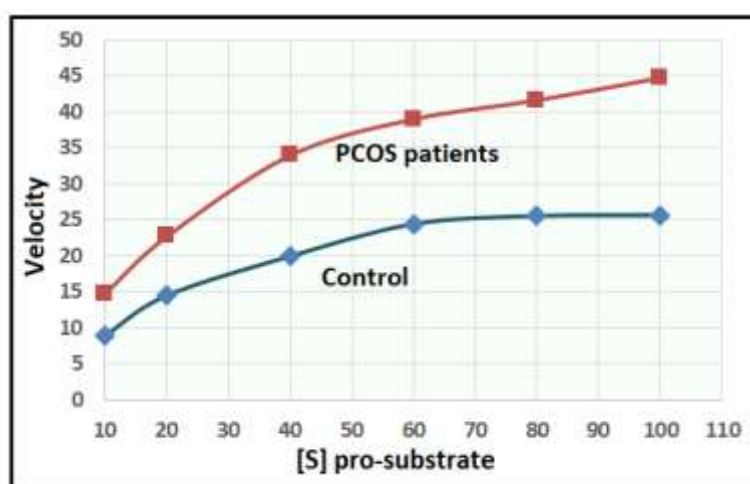


Figure 3: Kinetics of prolidase in RBC hemolysate of PCOS patients and controls. Michaelis Menten equation

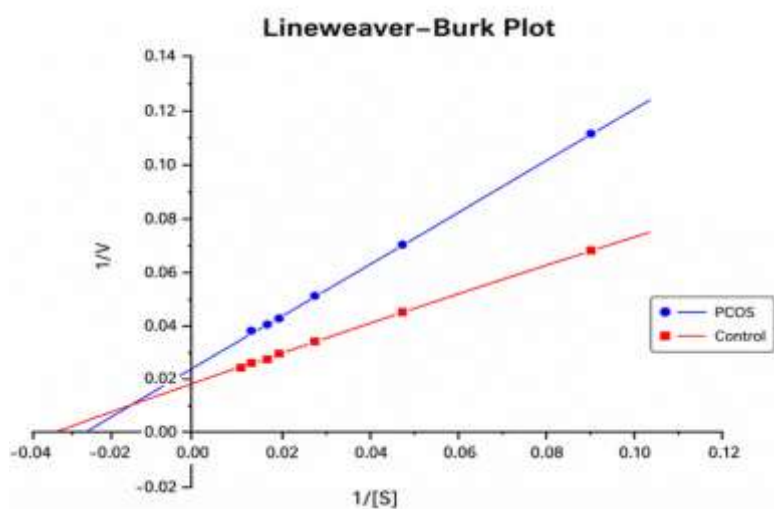


Figure 4: Kinetics of prolidase in RBC hemolysate of PCOS patients and controls. Line weaver-Burk plot

Table 5. kinetic studies of prolidase activity in plasma and RBC hemolysate in PCOS patients and control

Sample	Michaelis-Menten equation		Line weaver-Burk plot	
	Km (mM)	Vmax ($\mu\text{mol Pro min}^{-1} \text{mg}^{-1}$)	1/Km (mM)	1/Vmax ($\mu\text{mol Pro min}^{-1} \text{mg}^{-1}$)
P.C.	28.57	30.62	35.0×10^{-3}	32.66×10^{-3}
P.P	25.64	16.54	39.0×10^{-3}	60.46×10^{-3}
H.C.	29.41	58.01	34.0×10^{-3}	17.23×10^{-3}
H.T.	38.46	46.96	26.0×10^{-3}	26.0×10^{-3}

PC: plasma control, P.P: plasma patients, HC: Hemolysate control, H.P: hemolysate patients

DISCUSSION

The present study investigated alterations in plasma and erythrocyte prolidase enzyme kinetics in women with polycystic ovary syndrome (PCOS) to explore their potential role in extracellular matrix (ECM) remodeling and ovarian fibrosis. The findings demonstrated significant endocrine and metabolic abnormalities accompanied by distinct alterations in prolidase kinetic parameters, supporting the hypothesis that impaired collagen metabolism contributes to the pathophysiology of PCOS.

The demographic characteristics showed no significant differences in age or body mass index between women with PCOS and healthy controls, indicating that both groups were appropriately matched. This minimizes the potential influence of age and obesity on the observed biochemical and kinetic alterations and suggests that the differences are primarily attributable to PCOS itself. Similar observations have been reported in previous studies emphasizing that metabolic and reproductive disturbances in PCOS may occur independently of obesity [1–4].

Women with PCOS exhibited the characteristic endocrine profile of the syndrome, including significantly increased serum LH, testosterone, AMH, prolactin, estradiol, and LH/FSH ratio, together with significantly reduced progesterone concentrations. These hormonal abnormalities reflect chronic anovulation, excessive ovarian androgen production, and impaired follicular maturation, which are well-established features of PCOS [1–5]. Elevated AMH concentrations further indicate the accumulation of pre-antral and small antral follicles resulting from arrested follicular development.

The present study also demonstrated significantly higher fasting blood glucose, fasting insulin, and HOMA-IR values in women with PCOS, confirming the presence of insulin resistance as a major metabolic abnormality. Hyperinsulinemia enhances ovarian androgen production, suppresses hepatic sex hormone-binding globulin synthesis, and acts synergistically with luteinizing hormone to exacerbate hyperandrogenism and follicular dysfunction [5,18,20]. Furthermore, significantly increased total cholesterol, triglycerides, and LDL-cholesterol concentrations indicate the presence of dyslipidemia, which may further promote oxidative stress and chronic inflammation associated with PCOS.

One of the principal findings of the present study was the alteration of prolidase enzyme kinetics in both plasma and erythrocyte hemolysates. Plasma prolidase demonstrated a lower Km value accompanied by a marked reduction in Vmax compared with healthy controls. A lower Km reflects increased substrate affinity, whereas a reduced Vmax indicates diminished maximal catalytic activity. These findings suggest that although prolidase maintains effective substrate binding, its catalytic capacity is impaired, possibly because of persistent extracellular matrix remodeling and continuous collagen turnover associated with PCOS [10–14].

In contrast, erythrocyte prolidase exhibited an increased Km together with a reduced Vmax, indicating both decreased substrate affinity and impaired catalytic activity. The different kinetic behavior observed between plasma and erythrocyte compartments suggests that prolidase regulation may be influenced by the distinct metabolic environments of extracellular and intracellular compartments. Chronic oxidative stress, inflammation, and metabolic disturbances characteristic of PCOS may alter enzyme conformation and reduce its catalytic performance [11,15–17].

Extracellular matrix remodeling has emerged as a fundamental mechanism contributing to ovarian dysfunction in PCOS. Excessive collagen deposition, stromal expansion, and ovarian fibrosis interfere with normal follicular growth and ovulation. Because prolidase catalyzes the final step of collagen degradation, alterations in Km and Vmax observed in the present study

may reflect impaired collagen turnover and disturbed extracellular matrix homeostasis. These findings therefore support previous reports highlighting the importance of collagen metabolism in the progression of ovarian fibrosis associated with PCOS [6–10].

From a clinical perspective, evaluation of prolidase kinetic parameters provides functional information beyond conventional biochemical measurements. While enzyme concentration reflects the amount of prolidase present, kinetic parameters such as K_m and V_{max} characterize enzyme behavior and catalytic function. Assessment of these parameters may therefore improve understanding of extracellular matrix remodeling and contribute to the identification of functional biomarkers associated with ovarian fibrosis in women with PCOS [10,11,21–25].

Overall, the present findings demonstrate that prolidase enzyme kinetics are significantly altered in women with PCOS. The observed reductions in V_{max} together with changes in K_m indicate impaired enzyme function that may contribute to abnormal collagen turnover and extracellular matrix remodeling. These results support the involvement of prolidase dysfunction in the pathogenesis of PCOS and suggest that kinetic analysis may provide valuable functional biomarkers for evaluating ovarian extracellular matrix alterations. Further multicenter studies involving larger populations are warranted to validate these findings and to determine their potential clinical application.

CLINICAL IMPLICATIONS

The present findings provide further insight into the role of prolidase enzyme dysfunction in the pathophysiology of polycystic ovary syndrome. Evaluation of prolidase kinetic parameters, particularly the Michaelis constant (K_m) and maximum reaction velocity (V_{max}), offers functional information regarding enzyme activity beyond conventional biochemical measurements. The observed alterations in these kinetic parameters suggest disturbed collagen turnover and extracellular matrix remodeling, which may contribute to ovarian fibrosis and impaired follicular development.

Assessment of prolidase enzyme kinetics may therefore complement conventional hormonal and metabolic biomarkers in improving the understanding of disease progression. Furthermore, characterization of enzyme function may provide a useful approach for investigating extracellular matrix alterations and may support future therapeutic strategies targeting ovarian tissue remodeling in women with PCOS.

STRENGTHS OF THE STUDY

The present study has several strengths. First, it is among the few studies to investigate prolidase enzyme kinetics in women with PCOS by evaluating both plasma and erythrocyte hemolysates, providing a comprehensive assessment of enzyme function in two different biological compartments. Second, determination of the Michaelis constant (K_m) and maximum reaction velocity (V_{max}) using Michaelis–Menten and Lineweaver–Burk kinetic models enabled functional characterization of prolidase activity rather than measurement of enzyme concentration alone. Third, the inclusion of age- and body mass index-matched healthy controls minimized potential confounding factors. Finally, the study provides additional evidence supporting the association between altered prolidase kinetics, impaired collagen turnover, and extracellular matrix remodeling in women with PCOS.

CONCLUSIONS

The present study demonstrated significant alterations in prolidase enzyme kinetics in both plasma and erythrocyte hemolysates of women with PCOS. Plasma prolidase exhibited increased substrate affinity together with reduced maximum reaction velocity, whereas erythrocyte prolidase showed reduced substrate affinity accompanied by decreased catalytic capacity. These findings indicate that prolidase dysfunction in PCOS involves functional alterations in enzyme kinetics that may affect collagen metabolism and extracellular matrix homeostasis.

The observed changes in K_m and V_{max} support the hypothesis that impaired collagen turnover contributes to extracellular matrix remodeling and ovarian fibrosis in PCOS. Therefore, assessment of prolidase enzyme kinetics may provide valuable functional biomarkers for understanding disease mechanisms and may contribute to the future clinical evaluation of extracellular matrix abnormalities in women with PCOS. Further studies involving larger populations are recommended to validate these findings and explore their potential clinical applications.

Ethics Approval and Consent to Participate

The study protocol was approved by the Scientific and Ethical Committee of the College of Medicine, Al-Nahrain University, Baghdad, Iraq. Written informed consent was obtained from all participants before enrollment. All study procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

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