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RESEARCH ARTICLE

INVESTIGATION of ACTIVITIES ENZYME PROLIDASE (PRO) and GLUTATHIONE S-TRANSFERASE (GST) in POLYCYSTIC OVARY SYNDROME (PCOS) PATIENTS

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ABSTRACT

This study investigated serum antioxidant enzyme activity (Glutathione-S-transferase) and Prolidase in patients with PCOS.

A total of 42 patients with PCOS and 43 healthy control subjects were enrolled. Serum Prolidase and Glutathione-S-transferase (GST) activities were measured spectrophotometrically.

Age, TSH, fT4, Prolactin, Estradiol, FSH, systolic and diastolic blood pressure parameters were not statistically significant between PCOS group and healthy control group. BMI, weight, height, waist-hip ratio, menstrual cycle, FGS, FPG, LH, Insulin, androstenedione, SHBG, total testosterone parameters were statistically significantly increased in the PCOS group compared to the healthy control group. The serum glutathione-S-transferase was significantly decreased ($p < 0.05$) in patients with PCOS, compared with control subjects. Prolidase (Pro) activity has been found to be significantly higher in women with PCOS than in the control group.

Antioxidant enzyme GST activity has found to be decreased in PCOS patient group. Prolidase (Pro) enzyme is a candidate to be a leading parameter in the elucidation of the disease. In conclusion, the activities of glutathione-S-transferase and Prolidase (Pro) enzymes may be precursors in the etiopathogenesis of PCOS. This study has done in the literature for the first time. In addition, more work should be done in this area.

Keywords: *PCOS, Prolidase, Glutathione-s-transferase (GST)*

1. INTRODUCTION

Polycystic ovary syndrome (PCOS), which affects fertile women, is known as one of the endocrine disorders. This extended disorder of unknown aetiology is characterized by three fundamental properties: chronic anovulation, hyperandrogenism and ultrasonographic evidence of polycystic

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ovaries [1, 2]. In metabolism, antioxidants may decrease if oxidative stress increases. [3]. There are many different types of antioxidants [4,5]. Collagen is a very large component of extracellular matrix (ECM) [6]. The Glutathione-S-transferase (GST) has first described in 1961 by Booth et al. [7].Glutathione-s-transferase neutralizes electrophilic xenobiotics. Also, it is the dimeric enzymes that provide the excretion from the body [8,9] . Thanks to its antioxidant effects, the GST family is effective in the destruction of harmful reactive oxygen derivatives formed in the case of oxidative stress. It has been reported that changes in the genetic or structural structure of GST enzymes may cause many diseases, especially PCOS, in women [10]. Prolidase (E.C. 3.4.13.9) is involved in collagen synthesis and recovery of proline for cell growth, and also acts as an interface between protein nutrition and matrix breakdown [11]. Prolidase enzyme activity has been studied in various disorders, such as chronic liver disease; diabetic neuropathy, renal cell carcinoma, acute hemorrhagic stroke, osteoporosis, osteoarthritis, uremia and helicobacter pylori infection [12-19]. Collagen and extracellular matrix proteins are powerful in the regulation of cellular events, tissue stabilization and prevention of invasion. Matrix metalloproteinases (MMP) break down collagen, leading to the spread of diseases. Prolidase is active in matrix remodeling and collagen turnover, as it is a member of the MMP family [20]. It has stated in the study conducted by Wilk et al. that that increased prolidase levels in parallel with the abnormalities in collagen metabolism are associated with malignant diseases. It was determined that the microenvironment of the tumor was reorganized and its invasiveness increased thanks to the increased prolidase levels [21]. In a study conducted in endometrial cancers, it has found that prolidase levels increased and it could be associated with focal invasion [22]. Our aim is to reveal the relationship between enzymatic glutathione-S-transferase (GST) and prolidase (Pro) in PCOS.

2. SUBJECTS and METHODS

The study cases were enrolled from patients and healthy individuals presenting to the gynecology department in Van Training and Research Hospital.This study was performed on 42 (mean age of 24.2±5.9) women patients with PCOS and 43 (mean age of 23.7±4.4) healthy women volunteers with no history of PCOS. We received ethics committee approval for laboratory studies.

Blood of both patient and control groups were taken. Sera were obtained by separating them in a centrifuge at 5,000 rpm for 10 minutes. It was then stored at -85˚C.Determination of activity enzyme prolidase (Pro). Prolidase activity was performed according to the method developed by Myara et al. [23] . The definition of pcos is made according to the Rotterdam Criteria. These criteria include hyperandrogenism, oligoanovulation, and ultrasound image compatible with polycystic ovary morphology.[24]

2.1. Determination of Glutathione-S-transferase Activity (GST).

Glutathione S transferase activity (GST) has determined accordingto Habig et al., [25].

2.2. Statistical Analysis

Comparisons were made between groups. T-test was performed in the normal distribution condition was fulfilled. The Mann Whitney U test was performed when it was not meet the normal distribution condition. The Pearson correlation coefficient was calculated. Also, Spearman's rank correlation coefficient was measured. Significance level $p<0.05$ was considered significant. The data analysis was made in the SPSS (ver: 13) package program.

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3. RESULTS

Age, TSH, fT4, Prolactin, Estradiol, FSH, systolic and diastolic blood pressure parameters were not statistically significant between PCOS group and healthy control group. BMI, weight, height, waist-hip ratio, menstrual cycle, FGS, FPG, LH, Insulin, androstenedione, SHBG, total testosterone parameters were statistically significantly increased in the PCOS group compared to the healthy control group.
The serum Glutathione-s-transferase (GST) was significantly decreased ($p<0.05$) in patients with PCOS, compared with control subjects. Prolidase (Pro) activity has been found to be significantly higher in women with PCOS than in the control group.

Table 1. Parameters biochemical Glutathione-s-transferase and Prolidase (Pro).

	Control group (n=43)	**PCOS group (n=42)**	**P value**
Age(year)	23.7±4.4	24.2±5.9	0.311
BMI (kg/m2)	22.63±4.10	27.98±2.78	0.001
Weight(kg)	64.23±5.52	75.13 ±4.56	0.001
Height(cm)	165.34±6.43	160.23 ±4.10	0.001
Waist-hip ratio(cm)	0.66±0.17	0.78±0.15	0.001
SBP (mmHg)	115.12±11.33	122.20 ±6.60	0.324
DBP (mmHg)	75.10±4.46	78.13 ±4.12	0.231
Menstruel cycle (day)	22.11±6.2	66.45 ±14.16	0.001
FGS	5.67 ±1.01	12.17±5.34	0.001
TSH (uIu /dl)	1.54±0.77	1.76±1.21	0.546
T4(ng/dl)	14.4±1.28	15.7±1.47	0.332
E2(pg/ml)	47.23±9.23	48.6±11.34	0.340
FPG (mg/dl)	84.13±7.65	127.45±11.67	0.001
FSH (mu /ml)	5.2±1.68	4.8±0.98	0.237
LH (mu /ml)	4.56±1.45	8.45±1.96	0.001
Insulin (U/ml)	5.35±1.22	9.54±1.34	0.001
Androstenedion(ng/ml)	1.55±0.72	4.32±1.11	0.001
SHBG (nmol/l)	132.13±9.21	66.32±11.34	0.001
Total Testosteron (ng/ml))	1.29±0.48	4.45±1.35	0.001
Prolactin (ng/ml)	16.80±2.45	21.9±3.6	0.538
Pro(U/L)	58.616 ± 2.991	149.679 ± 30.591	0.001
GST(U/L)	0.0291 ± 0.0146	0.0044 ± 0.0028	0.001

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Table 2. Correlation between parameters in patient with PCOS.

	Prolidase	**GST**	**Androstenedion**	**BMI**	**E2**	**FGS**	**FSH**	**LH**	**Prolaction**	**Total Testesteron**	**TSH**	**Whr**	**Age**
Prolidase(U/L)	1												
GST(U/L)	0,064	1											
Androstenedion(ng/ml)	-0,067	0,093	1										
BMI(kg/m2)	-0,051	0,204	-0,154	1									
E2(pg/ml)	0,241	-0,066	0,211	-0,041	1								
FGS	0,114	-0,149	0,019	0,171	**0,420****	1							
FSH(mu/ml)	0,141	-0,023	**0,330***	0,021	0,106	**0,300***	1						
LH(mu/ml)	0,017	**0,343***	0,03	0,265	0,012	0,186	-0,147	1					
Prolaction(ng/ml)	-0,092	0,099	-0,026	-0,117	**-0,309***	**-0,302***	0,068	**-0,308***	1				
Total Testesteron(ng/ml)	0,038	0,12	**0,619****	0,178	0,193	0,079	**0,313***	-0,115	-0,143	1			
TSH(ulu/dl)	-0,122	0,077	0,062	0,083	-0,006	0,088	-0,1	**0,286***	**-0,283***	0,141	1		
Whr	0,022	-0,108	0,133	0,071	0,202	0,25	0,061	0,015	-0,213	0,021	0,268	1	
Age	-0,097	0,068	-0,122	**0,461****	0,128	0,252	-0,205	0,262	**-0,318***	0,004	0,151	-0,036	1

*Corelation is significant at the 0.05 level (2tailed)

**Corelation is significant at the 0.01 level (2tailed)

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34% between GST and LH, 33% between Androstendion and Fsh, 62% between Androstendion and Testesterone, 46% between BMI and age, 42% between E2 and FGS, 30% between FGS and Fsh,%31 between Fsh and Testesterone, 29% between LH and TSH positive, 31% between E2 and Prolactin, 30% between FGS and Prolactin, 31% between LH and Prolactin, 28% between Prolactin and TSH, 32% between Prolactin and age negative and statistically significant a significant correlation was found ($p < 0.05$).

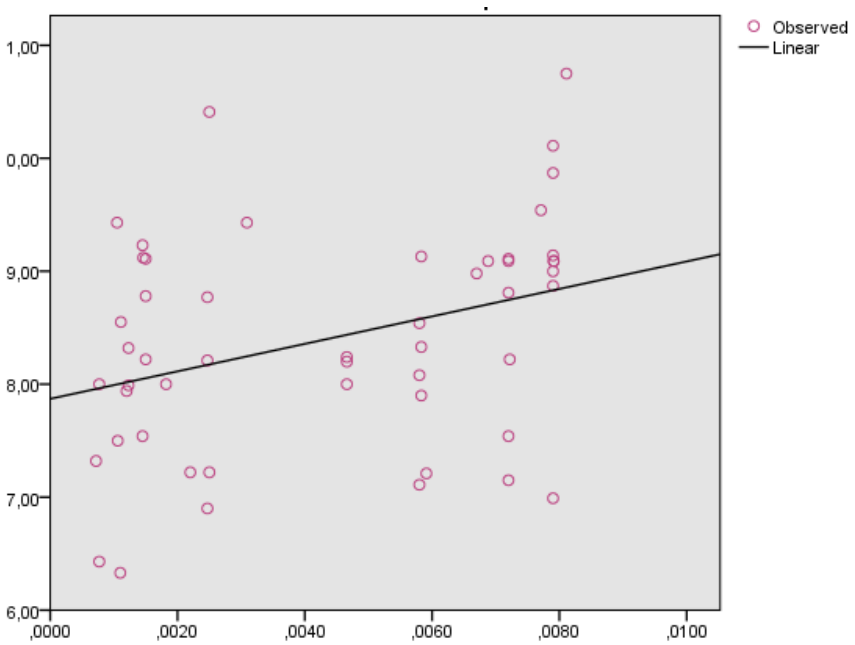

Figure 1. Correlation between LH and GST in patients with PCOS.

4. DISCUSSION

PCOS is an important problem especially for women between the ages of 15-45. This syndrome has the effect of causing DM, hypertension, infertility and cancer later in life.[26]. The exact cause of this disease, which has a complex pathophysiology, is still unclear. It has many causes such as insulin resistance, oxidative stress, metabolic syndromes and hyperandogenism. Today, the diagnosis and correct management of this disease is very important to prevent negative situations that may occur in the future.[27,28]. Although the place of oxidative stress in the pathophysiology of PCOS has been shown in many studies, this relationship between them has not been clarified yet [29]. In this study, we aimed to research the relationship of two parameters that contribute to the oxidative stress mechanism, such as prolidase and gst, with PCOS. PCOS is a very common disease in women [30,31].The definition of oxidative stress is the deterioration of the balance between oxidant and antioxidant [32].The level of oxidative stress have been increased in many studies and in different diseases [33].In patients with PCOS, oxidative stress increases and affects egg follicles [34].It has been suggested that oxidative stress parameters may be important in women with PCOS [35]. In case of difference in amount between oxidant and antioxidant molecules, an increase in harmful structures called reactive oxygen species (ROS) occurs. The occurrence of ROS has been found to be associated

with many ailments in women, especially PCOS, infertility and abortion. [36,37]. Due to the excessive amount of ROS in the body, irregularity may occur in the amount of cellular calcium ions. Due to this unbalanced situation, mitochonrial membranes and ATP metabolism are adversely affected, and in this case, it may contribute to menstrual irregularities and follicle development disorders in women.[38]. In contrast, cells have molecules called antioxidants that prevent these reactions by donating an electron to free radicals before they become unstable. Thanks to these important antioxidant enzymes, including GST, tissue damage is prevented in case of excessive ROS production in the cell [39]. The relationship between oxidative stress (OS) and PCOS has studied by Desai et al.
In a study of 25 non-obese PCOS and 25 healthy women. treatment has been found to be effective in preventing the progression of the disease [40]. In the study performed by Rahsepar et al., in which the relationship between vitamin D level and OS in women with PCOS was investigated, a total of 150 patients between the ages of 20 and 40 were included in the study. In this study, no significant difference was observed between the two groups in terms of serum 25(OH)D levels and oxidative stress [41]. The association of OS with malignant diseases in women has investigated in PCOS patients. It has been stated that ROS disrupts the DNA structure, causes point mutations and affects the basic mechanism of the body by affecting tumor suppressor genes. In the study conducted by Zuo et al., it has determined that there is a relationship between OS and DNA methylation and damage [42]. It has been shown that there is a clear association between women with PCOS and metabolic syndromes, and it has been stated that the risk increases especially as the BMI increases. It has been determined that increased fasting insulin level and BMI contribute to androgen production in the ovaries in patients with PCOS [43]. In our study, a significant difference was observed between the two groups in terms of BMI and Fasting Insulin levels. Looking at the literature, one of the studies investigating the links between PCOS and hyperandrogenism has conducted by Dunaif A et al. In our study, a significant difference was observed between the two groups in terms of SHBG, testosterone and androstenedione levels [44]. Glutathione - S - Transferase (GST) is a group of multiproteins hepatic and electrophilic chemicals that potentially remove harmful hydrophilic compounds from blood. Glutathione transferase (GST) detoxifies xenobiotics and free radicals, but also plays a role in catalyzing the conjugation of different endogenous substrates, including steroids andprostaglandins. Also, In addition, GST functions as binding proteins for steroid hormones, bile acids and neurotransmitters [45].As a result of hyperandrogenemia, it has been reported that the increase of free radical production in the development of PCOS [46,47].The serum glutathione-s-transferase was significantly decreased all ($p < 0.05$) in patients with PCOS, compared with control subjects. In this study, a positive correlation was also observed between GST and LH. In a study by Shenta et al., in which the effect of Gst on pcos patients has studied, it has found that GST levels, known for its antioxidant effect, were lower in pcos patients. [48]. In a study by Alves et al, in which GSTgen polymorphism has investigated and 201 patients participated, it has stated that there was no significant difference in terms of GST polymorphism between pcos patients and the control group [49]. In another study investigating the relationship between pcos and GST in the literature, lower GST levels have found in pcos patients. [50].

Prolidase (Pro)(EC.3.4.13.9) is a metalloprotease group [13]. It has a special requirement for manganese and ion (Mn+2) activates the enzyme [51].The final stage of collagen degradation is done by prolidasee [52].Prolidase has a unique role in all cell types .Prolidase enzyme activity has been suggested to be a speed limiting factor in the regulation of collagen biosynthesis [53].Prolidase enzyme activity has been found in erythrocytes and organ [54]. Prolidase plays an important role in many mechanisms for the regular functioning of cellular events. Thanks to the disintegrating feature of prolidase, proline or hydroxyproline, which plays an important role in intracellular communication, is formed. Prolidase is a ligand of human epidermal growth factor receptor 2 (HER2) and contributes

greatly to regeneration processes in case of tissue injury or infection under normal conditions. In particular, nuclear factor κβ (NF-κβ) contributes to the formation of an inflammatory response by affecting the transcription factor [55]. Prolidase enzyme activity has been studied in many diseases such as preeclampsia[56] postmenopausal osteoporos[57] ,early pregnancy loss [58], infertility and erectile dysfunction [59] and ovarian cancer [60]. Tuncay et al. 116 covid and 46 healthy patients participated in a study investigating the relationship between covid 19 and prolidase, which has recently emerged. As a result of this study, prolidase levels have found to be lower as a reflection of increased inflammatory mechanisms in covid patients and have associated with the inflammatory process [61].

In a study investigating the effects of oxidative stress factors and prolidase on unexplained infertility, prolidase levels have found to be higher in the patient group and it has stated that it has a place in pathophysiology [62]. There are few studies on prolidase in patients PCOS. In a study conducted by Hilali et al, which included 61 patients, it was found that serum Prolidase activity and oxidative stress levels were increased in women with PCOS compared to normal healthy group. Prolidase activity and oxidative stress level increased in PCOS patients [63].In another study performed by Bhatnager et al, Prolidase levels have significantly higher in patients with PCOS.Also, İt has found a significant correlation between increasing Prolidase level and ovarian cyst count. In our study, prolidase levels were found to be statistically significantly higher in the PCOS group compared to the control group [64].

As a result, antioxidant enzyme GST activity has found to be decreased in PCOS patient group. Prolidase (Pro) enzyme is a candidate to be a leading parameter in the elucidation of the disease. The activities of glutathione-S-transferase and Prolidase (Pro) enzymes may be precursors in the etiopathogenesis of PCOS. This study has done in the literature for the first time. In addition, more work should be done in this area.

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