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Evaluation of Glutathione-Related Antioxidant Enzyme Activity in Patients with Polycystic Ovary Syndrome (PCOS) and Investigation of Clinical Correlations

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Abstract: The aim of this study was to illustrate the importance of glutathione-associated enzymes as vital markers of antioxidant/oxidant activity in PCOS disease. Our study comprised a cohort of forty patients, all of whom were above the age of 18 and had been diagnosed with Polycystic Ovary Syndrome (PCOS) according to the 2003 Rotterdam diagnostic criteria. In addition, we recruited forty healthy individuals who were free from any metabolic disorders. The Rotterdam diagnostic criteria for polycystic ovarian syndrome consist of three main factors: oligo-anovulation, clinical and/or biochemical hyperandrogenism, and ultrasonographic confirmation of polycystic ovaries. Individuals who satisfied at least two of the three criteria were categorized as having Polycystic Ovary Syndrome (PCOS). The levels of gamma-glutamyl transferase (GGT) and delta-glutamyl cysteine synthetase (γ-GCS) in serum samples from both patients and controls were measured using the enzyme-linked immunosorbent assay (ELISA) technique. The study examined the levels of essential enzymes involved in glutathione metabolism in patients with PCOS. The enzymes displayed significant differences between PCOS patients and control groups, indicating that glutathione metabolism plays a pivotal role in the progression of this disease. After conducting correlation analysis, it is clear that enzymes have a reciprocal impact on the progression of the disease. The analytical findings suggest that there was no statistically significant disparity in GGT levels between the PCOS group and the control group ($p>0.05$). The levels of γ-GCS were significantly higher in the PCOS group compared to the control group ($P< 0.05$). Considering the involvement of γ-GCS in the process of glutathione catalysis, it is postulated that its concentrations would increase in response to an elevation in glutathione levels. Our analysis of the available evidence leads us to conclude that glutathione will exert a substantial influence on the mechanism of PCOS. Polycystic ovarian syndrome (PCOS) is a notable medical illness that frequently affects women and has a substantial influence on their overall well-being.

Keywords: Polycystic ovary syndrome, Antioxidant, Gamma-glutamyl transferase, Gamma-glutamyl cysteine synthetase

Introduction

Polycystic ovary syndrome (PCOS) is a medical disorder characterized by ovarian dysfunction, elevated levels of male hormones (hyperandrogenism), and the presence of several cysts in the ovaries as observed through ultrasound imaging (multicyclic ovarian morphology) (Fathi, 2023). Although it is the most common hormonal problem among women of reproductive age, the precise origin of this disease remains incompletely understood

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(Baqer et al., 2017). Nevertheless, it is hypothesized that the present expression of the illness arises in individuals with a susceptible genetic predisposition, which is altered by specific environmental factors (Trent & Gordon, 2020). Polycystic ovarian syndrome has a wide array of clinical symptoms that are diverse and varied. Elevated levels of androgens in the bloodstream are indicative of hyperandrogenism. In addition, it can present itself through dermatological symptoms such as hirsutism (abnormal hair growth), acne, and androgenic alopecia (male pattern hair loss). Ovarian failure commonly manifests as infrequent menstrual periods, known as oligomenorrhea, and can ultimately result in the inability to conceive, also known as infertility. Individuals with Polycystic Ovary Syndrome (PCOS) often encounter metabolic complications such as insulin resistance and obesity (Melkiyur et al., 2023). Studies have shown that women diagnosed with polycystic ovarian syndrome have an increased vulnerability to various mood and anxiety disorders, as well as problems associated to their endocrine and metabolic systems (Cunha & Póvoa, 2021 ; Buczinski et al.,2023) observed a correlation between the occurrence of acne and depression (Buczinski et al., 2020). In addition, a study carried out in Turkey showed hirsutism and monthly irregularity as notable risk factors for patients, as reported by Chen et al. (2020). In 1990, the National Institutes of Health (NIH) created specific guidelines for diagnosing polycystic ovarian syndrome (PCOS). The criteria for diagnosis include the presence of clinical/biochemical hyperandrogenism and recurrent irregular anovulatory menstrual cycles. However, it was later found that the two criteria set by the National Institutes of Health (NIH) may not be sufficient for diagnosing polycystic ovarian syndrome (PCOS) in later years. Research has discovered that individuals with less severe features, such as normal levels of androgens and regular ovulation, can also experience PCOS, as shown by ultrasonographic evidence. Following that, PCOS was categorized into four separate clinical phenotypes according to the Rotterdam criteria, which were published in 2003 by Esher and ASRM in 2004. Serum gamma-glutamyl transferase (GGT) and gamma-glutamyl cysteine synthetase (γ-GCS) play a role in the oxidant/antioxidant system of eukaryotes. The aim of this study was to enhance our comprehension of the progression of the disease by measuring the quantities of crucial enzymes involved in glutathione metabolism in women diagnosed with PCOS and healthy volunteers.

Materials and Methods

The study was conducted in accordance with the principles described in the World Medical Association Declaration of Helsinki, after obtaining approval from the ethics committee. The study was undertaken independently, without any financial support.

Patients' Inclusion Criteria

Our study involved a sample of forty adult patients who were diagnosed with polycystic ovarian syndrome (PCOS) based on the 2003 Rotterdam diagnostic criteria. In addition, we recruited forty healthy individuals who did not have any metabolic conditions to serve as a control group. The Rotterdam diagnostic criteria for polycystic ovarian syndrome consist of three main factors: oligo-anovulation, clinical and/or biochemical hyperandrogenism, and ultrasonographic confirmation of polycystic ovaries. Individuals who satisfied at least two of the three criteria were categorized as having Polycystic Ovary Syndrome (PCOS).

Studying Parameters

The quantification of GGT and γ-GCS was performed using the double sandwich ELISA technique. The enzyme-linked immunosorbent test (ELISA) is largely recognised as the most dependable technique for identifying disease-associated biomarkers in clinical laboratories worldwide. Neurological disorders, malignancies, and inflammatory diseases can be diagnosed with ELISA tests that are available for purchase. Subtle nuances in facial expression can facilitate the identification of many biomarkers linked to illness. The enzyme-linked immunosorbent assay (ELISA) was developed from a radioimmunoassay (RIA) created by Hoffman (1973). In a traditional ELISA, antibodies are used to fix antigens onto an ELISA plate, which acts as a rigid support composed of plastic. Afterwards, an enzyme is used to convert the substrate into a detectable signal. The level of the antigen is directly proportional to the strength of the signal (Klumpp Thomas et al., 2021). Thus, ELISA can be employed to detect the antigen and ascertain its quantity. The procedure entails the step-by-step introduction of ELISA components onto the test plate, followed by a period of incubation and subsequent analysis of the obtained results. An ELISA assay has several crucial elements, namely antigen, substrate, secondary antibody, enzyme-conjugated antibody, and primary antibody. During ELISA, various buffers are used to dilute the components, eliminate any surplus chemicals, occupy any vacant areas on the

plate, and terminate the substrate reaction. Over the course of time, various iterations of ELISA have been created in order to enhance the assay's specificity, decrease interference, and enhance sensitivity. The five predominant types of these procedures comprise direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA, and ELISA utilising nanoparticles. The interaction between antigens and antibodies remains constant in all ELISA formats; however, the order and quantity of steps involved may vary (Williams et al., 2016).

Gamma-Glutamyl Transferase (GGT)

The samples, kit reagents, and microplate utilized in the investigation were allowed to equilibrate to room temperature. The concentration of human GGT in the samples was determined using the enzyme-linked immunosorbent assay (ELISA) technique, with the aid of a kit provided by Bioassay Technology Laboratory (Catalogue No: E6022Hu, China). This kit uses the biotinylated double sandwich method for measurement. This kit uses a microplate that has been pre-coated with a purified rat monoclonal GGT antibody. 50 µL of GGT standards (2400, 1200, 600, 300, and 150 ng/L) and 40 µL of samples were added to the wells. A volume of 10 microliters of biotinylated anti-GGT antibody was added to the samples. Subsequently, 50 µL of streptavidin-HRP was added to both the samples and standards. Subsequently, the mixture was transferred to a Sanyo Sterilizer incubator, a device manufactured in Japan, and kept at a constant temperature of 37 °C for a period of 1 hour. After the incubation period ended, the ELISA (Biotek ELx50, USA) was cleaned using a specialized washing apparatus. Afterwards, 50 µL of Chromogen A and Chromogen B solutions were added and placed in a light-restricted environment at a temperature of 37 ^{o}C for 15 minutes. The reaction was stopped by putting it to darkness to enhance color formation, and then introducing an acidic solution. The color intensity was quantified at a wavelength of 450 nm using an ELISA reader (Biotek ELx800, USA). The GGT levels were assessed using conventional graphical techniques (Gnawali et al., 2021).

Gamma-Glutamyl-Cysteine Synthetase (γ-GCS)

The samples, kit reagents, and microplate designated for the enquiry were allowed to equilibrate to the surrounding temperature. The amount of human γ-GCS (Bioassay Technology Laboratory, Catalogue No: E6022Hu, China) in the samples was measured using an Enzyme Linked Immunosorbent Assay (ELISA) kit. This kit uses the biotinylated double sandwich method for measurement. This kit employs a microplate that has been pre-coated with a highly pure rat monoclonal gamma-glutamyl cysteine synthetase (γ-GCS) antibody. 50 µL of gamma-glutamyl cysteine synthetase (γ-GCS) standards (2.5, 1.25, 0.625, 0.312, and 0.156 ng/L) and 40 µL of samples were added to the wells. The samples were exposed to 10 microliters of biotinylated anti-gamma-glutamyl cysteine synthetase (γ-GCS) antibody. Subsequently, 50 µL of streptavidin-HRP was added to both the samples and standards. Subsequently, the mixture was transferred to a Sanyo Sterilizer incubator, a device manufactured in Japan, and kept at a constant temperature of 37 °C for a period of 1 hour. After the incubation period, the ELISA (Biotek ELx50, USA) was washed using the washing equipment. Afterwards, 50 µL of Chromogen A and Chromogen B solutions were added and placed in a light-free environment at a temperature of 37 oC for 15 minutes. The reaction was stopped by putting it to darkness to enhance color formation, and then introducing an acidic solution. The color intensity was quantified at a wavelength of 450 nm using an ELISA reader (Biotek ELx800, USA). γ-GCS levels were measured using traditional graphical methods (Muraoka et al., 2022).

Statistical Analysis

The data collected from our enquiry was examined using the SPSS 22.0 statistical software. The data collected prior to analysis and evaluation was assessed for conformity to a normal distribution using the Kolmogorov-Smirnov test. The Kolmogorov-Smirnov test was employed to compare measurements acquired from numerous distinct groups that were not influenced by each other. If the analysis demonstrated statistical significance, a one-way analysis of variance (ANOVA) was employed. Subsequently, pairwise comparison approaches were utilized to ascertain the distinct group or groups that exhibited differences. If the assumptions necessary for parametric testing were not satisfied, the Kruskal-Wallis test was used to compare results from many groups, independent of the variable being measured. The Kruskal-Wallis test is employed to ascertain the statistical significance of a decision through analysis. The Man-Whitney U test is thereafter employed to pinpoint the particular group or groups accountable for the observed disparity. The Chi-square test is used to evaluate qualitative data obtained from a census. The researchers considered a significance threshold of $p<0.05$ to be acceptable for statistical analysis (Mohr et.al.,2021).

Results

This study included a total of 40 patients who received a diagnosis of Polycystic Ovary Syndrome (PCOS) and 40 healthy volunteers who were matched in terms of age. After evaluating the demographic data of both sick and healthy individuals, no statistically significant difference was seen in terms of age (p>0.05). Table 1.

Table 1. Demographic data in patients and controls

Variables	PCOS (n=40)	Control (n=40)	p
Age (Year's)	31,28 ± 5,43	30,38 ± 4,33	0,745
p<0,05 is statistically important value			

The study examines the levels of Serum GGT and γ-GCS in both PCOS patients and the control group, as presented in Table 4.2. The statistical analysis showed that the levels of GSS and γ-GCS were significantly higher in the PCOS group compared to the other groups (p<0.05). Table 2

Table 2. Comparison of biomarkers in groups

	PCOS (n=40) Mean ± SD	Control (n=40) Mean ± SD	p
GGT	49,53 ± 17,01	51,05 ± 18,30	0,702
γ-GCS	0,228 ± 0,192	0,122 ± 0,126	0,006*
p<0,05 is statistically important value			

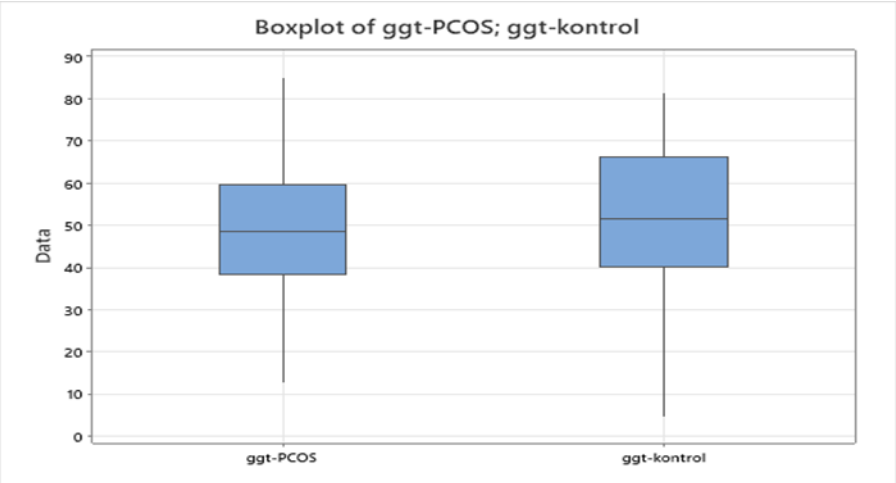

Figure 1. Boxplot graphic of GGT

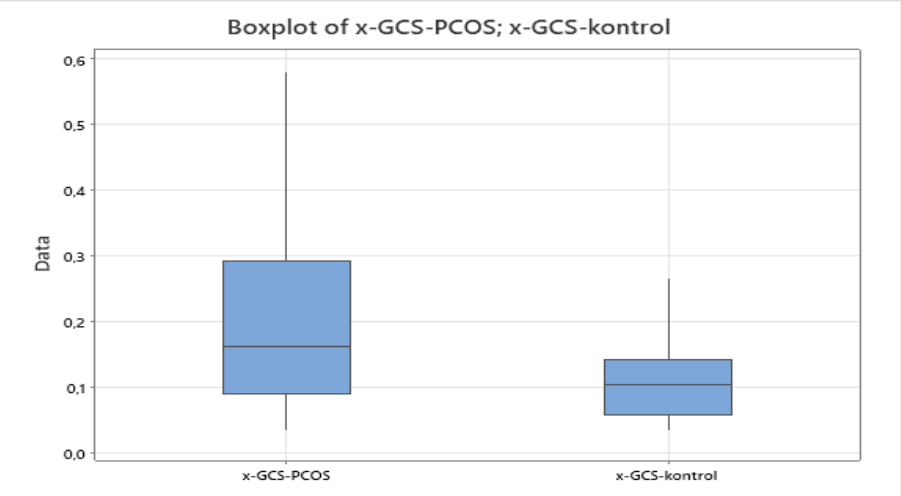

Figure 2. Boxplot graphic of GCS

Spearman's rho correlation analysis was performed to investigate the relationship between parameters in both the control and patient groups. Tables 3 and 4.

Table 3. Correlation analyzes for PCOS group

Spearman's rho		GGT	γ-GCS
GGT	r	1,000	0,226
	p		0,160
γ-GCS	r	0,226	1,000
	p	0,160	
p<0,05 is statistically important value			

A statistically significant and weak positive connection (p:0.160 and r:0.226) was identified between GGT and GCS in the group of individuals with PCOS.

Table 4. Correlation analyzes for control group

Spearman's rho		GGT	γ-GCS
GGT	r	1,000	0,724
	p		0,001*
γ-GCS	r	0,724	1,000
	p	0,001*	
p<0,05 is statistically important value			

A significant and strong positive correlation was observed between GGT and GCS in the control group. Polycystic ovarian syndrome is a medical illness that is defined by the presence of many physical characteristics and its effects on different body systems. The diagnosis is determined using a comprehensive set of 40 criteria that include clinical, biochemical, and imaging methods (Ramos et al., 2017). The study encompassed participants who were diagnosed with Polycystic Ovary Syndrome (PCOS) as well as persons who were in a state of excellent health. The individuals diagnosed with polycystic ovarian syndrome were identified based on the 2012 criteria established by the National Institutes of Health. The 2012 NIH standards adopted the 2003 Rotterdam diagnostic criteria as the official criteria for diagnosing PCOS. The diagnostic criteria for this disorder include oligo-anovulation, the presence of clinical and/or biochemical hyperandrogenism, and an ultrasonographic observation of polycystic ovary appearance (characterised by an ovarian volume greater than 10 cm^3 or the presence of more than 12 antral follicles) (Dybciak et al., 2022). In order to receive a diagnosis for this illness, an individual must meet a minimum of two out of these three criteria. The aim of our study was to measure the levels of key enzymes with antioxidant properties in persons diagnosed with PCOS, and to examine their influence on the development and advancement of the condition. To accomplish this goal, the levels of GGT and γ-GCS were measured using the ELISA technique in serum samples obtained from the subjects. After analysing the ages of both the patients and healthy volunteers, it was seen that there was no statistically significant difference. Enzyme levels and functions may vary depending on an individual's age. The absence of age gap between the groups indicates that our findings were not affected by age. After analysing our results, we found that the levels of γ-GCS were considerably higher in the PCOS group compared to the control group. Sonino et al. (1993) did a study which found that there was no notable disparity in GGT levels between the two groups (Sonino et al., 1993).

No studies have been undertaken to investigate the effects of glutathione-associated enzymes on the group with polycystic ovary syndrome (PCOS). Therefore, our study is important in clarifying the relationship between PCOS and the enzymes involved in the glutathione route. There is a significant amount of study in scientific literature that examines the relationship between Polycystic Ovary Syndrome (PCOS) and the interaction between antioxidants and oxidants. We examined the influence of glutathione-associated enzymes on the development of PCOS pathophysiology. Research has shown that levels of γ-GCS increase in patients with PCOS, possibly as a result of higher levels of oxidants. This may lead to an increase in antioxidants as a protective response (Tsiasioti & Tzanavaras, 2020).

Conclusion

We conducted a study to examine the levels of essential enzymes involved in glutathione metabolism in individuals diagnosed with polycystic ovarian syndrome (PCOS). The enzymes in individuals with polycystic ovary syndrome (PCOS) were significantly different from those in control groups, indicating that glutathione metabolism plays a vital role in the progression of this condition. After conducting correlation analysis, it is clear that enzymes have a reciprocal impact on the progression of the disease. Polycystic ovarian syndrome (PCOS) is a notable medical illness that frequently affects women and has a substantial influence on their

overall well-being. Therefore, it is essential to better elucidate the fundamental factors contributing to the illness.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM Journal belongs to the authors.

* Permission was obtained from the Tikrit University Local Ethics Committee for this study (Date: 05.04.2021, No: 2021.03/73).

Acknowledgements or Notes

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