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Assessing the Activity of Renin and GST in the Serum of Ladies Suffering from Polycystic Ovary Syndrome and COVID-19 to Predict the Danger of Cardiac Disease

Shaimaa Emad Ali* and ***Fayhaa M. Khaleel***

Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

The coronavirus-pandemic has a major impact on women's-mental and physical-health. Polycystic-ovary-syndrome (PCOS) has a high-predisposition to many cardiometabolic-risk factors that increase susceptibility to severe complications of COVID-19 and also exhibit an increased likelihood of subfertility. The study includes the extent of the effect of COVID-19-virus on renin-levels, glutathione-s-transferase-activity and other biochemical parameters in PCOS-women. The study included 120 samples of ladies that involved: 80 PCOS-patients, and 40 healthy-ladies. Both main groups were divided into subgroups based on COVID-19 infected or not. Blood-samples were collected from PCOS-patients in Kamal-Al-Samara Hospital, at the period between December until June. Some biochemical parameters were measured for all study-groups, which included: determination of serum renin levels by using the ELISA-technique, GST-activity, lipid-profile. FBS was assessed manually, and hormones were assessed using VIDAS-analyzer-hormones. The result showed a possible relationship between FBS-levels and renin in PCOS-ladies (Stein-Leventhal-Syndrome), while GST-activity were inversely associated with BMI in PCOS-ladies. Also, it was found that the renin-levels were higher in PCOS-patients groups compared with healthy-groups. On the other hand, the renin levels and Glutathione-S-Transferase-activity were lower in PCOS-patients-infected-with-COVID-19 than female-patients-without-COVID-19. The statistical-data-displayed that the level of renin is associated negatively with glutathione-s-transferase-activity in PCOS-cases. Renin level was higher in PCOS-ladies, this may lead to increase renal-dysfunction and risk of cardiovascular-disease that may be expected in patients. A decrease in the antioxidant-capacity may be because the high number of free-radicals that enter the body by the virus and high levels of renin which lead to a higher risk of PCOS-complication like cardiovascular-disease.

Keywords: Angiotensin-converting enzyme 2, Body mass index, COVID-19, Glutathione-S-Transferase, Polycystic ovary syndrome.

Introduction

Polycystic ovary condition (PCOS) is a typical endocrine problem influencing 5-13 of premenopausal ladies. The clinical appearances of hyperandrogenism (hirsutism, skin inflammation and scalp going bald) and feminine unsettling influence are well recognized. Conversely, the relationship with longer term unfriendly wellbeing results is less valued, regardless of an abundance of proof highlighting an expanded chance of metabolic and nonmetabolic morbidities[1]. Serious intense

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respiratory condition Covid 2 (SARS-CoV-2), answerable for COVID-19, has up until this point tainted more than 94 million and end life of about 1.8 million individuals around the world. The expanded hospitalization and mortality connected to COVID-19 are related with more age and various fundamental circumstances like heftiness and related comorbidities [2]. Weight appears to increase the chance of both COVID-19 complexities and mortality [3,4]. The expanded risk of fat individuals to create serious COVID-19 indications can be credited to different expected instruments, for example, the constant foundational phlogistic express, the useless and postponed insusceptible reaction, and, surprisingly, the fat tissue itself, which addresses a repository for the infection. Ladies' ovary disorder takes a part in elements metabolic condition, such as insulin hormone obstruction and weight, and also increase the extent proceeding to the foster Type 2 diabetes [5]. Hence, the cardio-metabolic sicknesses ordinarily found in ladies with PCOS cross-over with risk factors inclining toward serious COVID-19 illness [6]. Renin is secreted by granulocytes of the kidneys, the precursor of renin, called prorenin, is a 406-amino-acid long protein, and its processing constitutes the active protein. Prorenin can be proteolyzed in the kidney by neuroendocrine adapter 1 or cathepsin B, and is not proteolyzed in many tissues by renin/prorenin receptors. Renin in its active form contains 340 amino acids [7]. It occurs in multiple isoforms, with antagonistic functions. This enzyme is also considered a hormone for its signaling role. Arterial hypotension, decreased sodium chloride, and sympathetic nervous system activity lead to its release which is controlled by renin enzyme from the juxtaglomerular cells in the kidney and also can be released from the receptors on the wall of vascular and can be released from the sympathetic nerve [8]. GST is a large family of enzymes that catalyzes the development of the high conjugates of glutathione (GSH) with different type of xenobiotic substrates. These enzymes are necessary for the metabolism of damage electrophilic compounds. Also, it is responsible for the oxidative stress response. This enzyme removes the toxins from the drug compounds, carcinogens, in addition to its ability to inhibit tissue damage caused by oxidizing agents [9]. The cytosolic GSTs are the most complex family from these eight subdivisions or classes [9,10]. The aim of the study is to understand the effect of COVID-19 in PCOS patients by the estimation the levels of renin and GST and the correlation between parameters in patients and healthy groups infected or not infected with COVID-19.

Materials and Methods

Patients and Control: ten milliliters of blood sample were collected in gel tube (serum separated tube) from PCOS patients and healthy group infected or not infected with COVID-19 for renin test, ACE2, GST activity, D-dimer, lipid profile, and hormones (FSH, LH, testosterone). The circumference of the waist to the hip was measured by measurement tool, and the body mass index was also measured by the formula (weight / (Hight)^2).

Serum Sample Preparation: by letting the blood tube for 20-25 minutes at room temperature 25°C, then the sample was centrifuged at 2000-3000 rpm for 10 minutes. The separated serum was stored in deep freeze -20°C, in Kamal-Al Samara Hospital / laboratories department for the subsequent biochemical tests as the followings: - a number of 120 objects included PCOS patients 80 which was divided in 2 groups according to infection or non-infection with COVID-19. The first group was PCOS ladies infected with COVID-19 n= 40 and the second group was PCOS ladies non-infected with COVID-19 n= 40, and control group 40 also divided in 2 groups according infection or non-infection with COVID-19. The first group was healthy ladies infected with COVID-19 n= 20 and the second group was healthy ladies non-infected with COVID-19 n= 20. In this study, ladies (patients and healthy people) who had previous operations such as removal of the ovaries, removal of the uterus or fallopian tubes were excluded. Also, pregnant ladies or those with one of the following diseases were excluded: - (high blood pressure, cardiac issue, T2DM, chronic or acute kidney disorder).

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Statistical Analysis

All statistical analyses were performed using SPSS software version 26.0. Data with normal distribution was presented as mean ±SD and analysis of variance (ANOVA) as post hoc pairwise comparison (between more than 2 groups). A p-value ≤ 0.05 was put as a statistical signal refer to (significant difference).

Results and Discussion

The hormones were measured for a group of patients and healthy (control) ones 80 ladies suffering from PCOS infected and non-infected with COVID-19 and 40 control infected and non-infected with COVID-19. The results showed a highly significant difference p≤0.05 among all groups in regards to luteinizing hormone (LH), follicle stimulating hormone (FSH), LH/FSH, and testosterone. The results indicated that the level of all hormones (except hormone FSH) in the serum in the infected and uninfected patients with COVID-19 was significantly higher than the healthy groups at p≤0.01, as shown in Table 1.

Table 1. (mean ± SD) of Levels of hormones (FSH, LH, Testosterone) between study groups.

Groups Parameters	Polycystic ovary syndrome infection with COVID-19 Group	Polycystic ovary syndrome without infection with COVID-19 Group	Healthy infection with COVID-19 Group	Healthy without infection with COVID-19 Group	p-value
FSH **mIU/ mL**	6.22±2.41 2.30-12.9	4.87±2.22 2.10-16.3	8.50±2.20 4.20-11.57	7.70±2.91 3.50-15.97	**0.0001
LH **mlU/ mL**	9.18±3.13 1.95-16.2	8.69±5.55 3.10-36.5	5.09±2.12 2.10-9.34	4.44±1.97 2.07-9.70	**0.0001
LH/FSH ratio	1.56±0.46 0.47-2.65	1.86 ±0.98 1.0-6.58	0.763±0.76 0.24-3.90	0.58±0.16 0.33-0.88	**0.0001
Testosterone **Ng/ mL**	0.78±0.22 0.30-1.30	0.67±0.21 0.15-1.05	0.45±0.22 0.10-0.99	0.30±0.10 0.10-0.50	**0.0001

Anthropometric data of the participants as shown in Table 2, the mean ± standard deviation values of lipid profile for the studied groups showed a highly significant p≤0.05 in cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and very-low-density lipoprotein (VLDL) levels. Meanwhile the levels of low-density lipoprotein (LDL) showed a significant difference p≤0.05 between all groups.

Table 2. (mean ± SD) of lipid profile between study groups.

Groups Parameters	Polycystic ovary syndrome infection with COVID-19 Group	Polycystic ovary syndrome without infection with COVID-19 Group	Healthy infection with COVID-19 Group	Healthy without infection with COVID-19 Group	p-value
Cholesterol **mmol/L**	7.21±1.41 4.21-9.61	6.47±1.29 4.90-9.60	5.52±1.67 3.58-8.50	3.74±1.14 2.10-7.10	**0.0001
Triglycerides **mmol/L**	6.36±2.27 2.90-9.68	4.26±1.73 2.19-11.0	3.98±1.32 2.0-6.8	0.85±0.57 0.40-2.90	**0.0001
HDL **mmol/L**	1.04±0.59 0.15-2.77	1.08±0.45 1.20-2.88	1.10±0.82 0.20-2.70	1.33±0.39 0.86-2.14	**0.0001
LDL **mmol/L**	3.27 ±1.31 0.15-5.58	2.89±1.55 0.28-6.04	2.65±1.75 0.19-6.77	2.04±0.94 0.60-4.40	*0.020
VLDL **mmol/L**	2.89±1.03 1.31-4.40	1.78±0.63 0.90-3.55	1.76±0.64 0.80-3.09	0.38±0.26 0.18-1.31	**0.0001

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As shown in Fig.1, the proportion of insulin and HOMA IR basically higher in the patient ladies' groups infected or non-infected with COVID-19 compared with control groups $P \leq 0.05$.

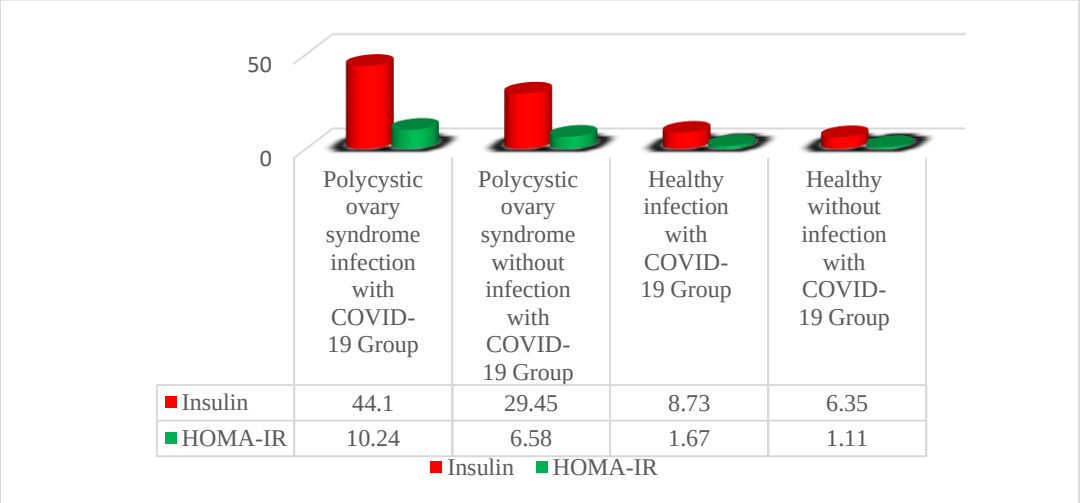

Figure 1. insulin and HOMA IR in patients with PCOS and control groups.

As shown in Table 3, the results indicted a statistically significant differences $p \leq 0.05$ in the serum renin in unit (pg/mL) between patients' groups PCOS patients infected with COVID-19, and PCOS patients non-infected with COVID-19 and healthy groups healthy infected with COVID-19, and healthy non-infected with COVID-19 were 163.93±24.22 pg/mL, 190.95±27.56 pg/mL, 97.079±4.005 pg/mL, and 101.80±3.08 pg/mL respectively. Also, the current study showed highly significant differences $p \leq 0.05$ in GST levels in unit (IU/L) between all study groups PCOS patients infected with COVID-19, and PCOS patients non-infected with COVID-19, healthy infected with COVID-19, and healthy non-infected with COVID-19 were 3.76± 2.41 IU/L, 5.05± 4.80 IU/L, 6.31± 5.83 IU/L, and 12.93± 7.81 IU/L respectively.

Table 3. (Mean ± SD) Levels of Renin and GST enzyme activity in PCOS groups and controls.

Groups / Parameters	Polycystic ovary syndrome infection with COVID-19 Group	Polycystic ovary syndrome without infection with COVID-19 Group	Healthy infection with COVID-19 Group	Healthy without infection with COVID-19 Group	p-value
Renin pg/mL	163.93±24.22	190.95±27.56	97.079±4.005	101.80±3.08	**0.0001
	123.06-210.60	136.15-237.16	91.35-105.18	94.31-106.35	
GST Activity IU/L	3.76± 2.41	5.05± 4.80	6.31± 5.83	12.93± 7.81	**0.0001
	0.52- 9.37	0.52- 24.12	1.04- 23.43	0.52-28.12	

The ROC curve analysis showed that the cut off points for renin and GST are > 106.35, ≤ 9.13 respectively and sensitivity 80.8 %, 91.9% respectively, and specificity 100%, 66.7%. The results displayed that renin and GST possess a perfect ability according to the area under the curve (AUC) for renin and GST are 0.903, 0.799 respectively to identify PCOS patients with COVID-19 from healthy controls, as shown in Fig.2. and Fig.3. The significance level is a very important $P<0.01$.

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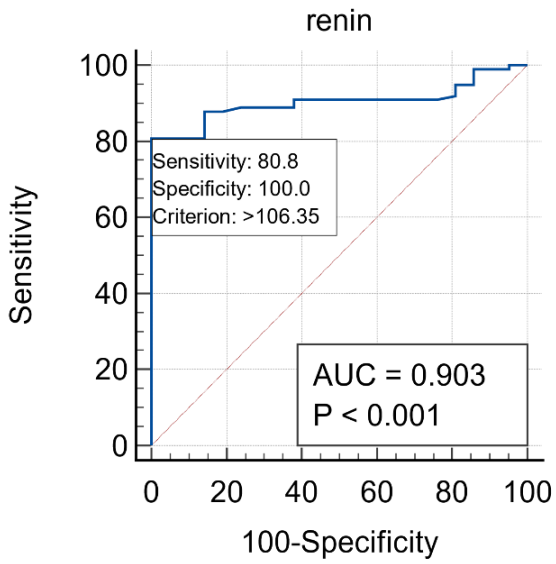

Figure 2. ROC curve of renin in PCOS patients.

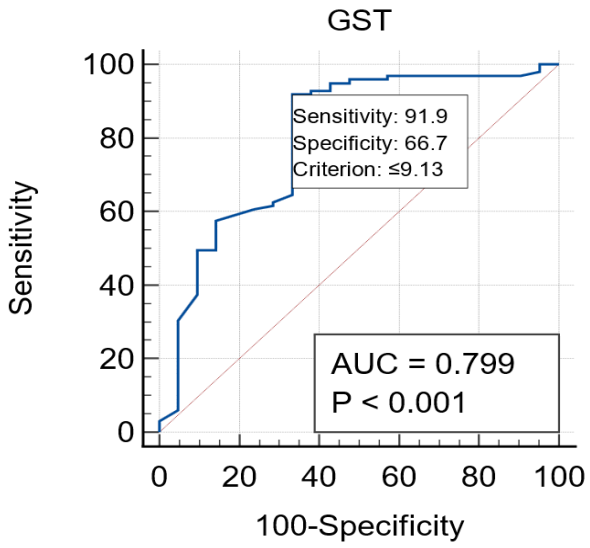

Figure 3. ROC curve of GST in PCOS patients.

Discussion

PCOS is the illness highlighted by raised degrees of androgens, ovulatory brokenness, and morphological anomalies. As indicated by national institutes of health, it tends to be characterized as "hyperandrogenism with ovulatory brokenness." At regenerative phase of the ladies, the pace of polycystic ovary event is estimated as 14 % [11]. The renin enzyme, is orchestrated in the renal and external tissue of the kidney like adrenal organs, ovary, testis, and pituitary gland. Prorenin is found in the mature ovary's follicles of female in highly levels. Prorenin but not renin enzyme increases at center monthly period soon next the luting hormones flood[12]. GST is a detoxifying protein fundamental for a cell insurance against oxidative harm [13]. In the current study, the levels of renin were found to ascend in PCOS patients, when compared with control groups. Additionally, it has been observed that the degrees of renin were lower in PCOS ladies tainted with COVID-19 more than non- infected patients. This outcome might be because of the idea of life, including mental and anxious tensions, notwithstanding the expansion in calories that an individual gets from sugars and fats, and food that contains a high level of sodium and potassium, and the association of this with latency, absence of development and exercise are the ways of knowing the mystery of hypertension. These results are in agreement with Moin, A. et al. [14] who found an increase in renin level in female patients in comparison with control groups. In the current study, it has been observed that glutathione s transferase activity decreased in ladies suffering from the syndrome compared with control groups. These results agree with Sun et al.,[15]. It has been observed that action of compound (GST) decreased in obese ladies with PCOS more than control group. The diminished movement of this cell reinforcement chemical in PCOS patients might be because of the expanded creation of oxidative stress in the ladies with PCOS because of expanded bad fat in the body prompting a risk of CVD and cardiovascular failure. These results are in agreement with Moti, et al.[16], who proved the expanded oxidized pressure, also the diminishing in the degree of cancer prevention agents in patient ladies when compared with control groups. In the current study, it was found that renin decreased in patient ladies infected with COVID-19 when compared with the non-infected patient. The result agrees with White, Melissa C., et al. [17], who found that renin level decreased in PCOS patients infected with COVID-19 compared with healthy groups. It showed that estrogenic action diminished the vascular reaction (vasoconstriction and NADPH oxidase activation) to Ang2 and worked to activate of ANG 1-7. Likewise, estrogen increments angiotensinogen, AT2 receptors, and ANG 1-7 yet diminished renin levels, ACE action, AT1 receptor thickness, and aldosterone. In the current study, pituitary hormones levels were found to increase except in FSH which decreased in patients with

PCOS and infected with COVID-19, when compared with the healthy groups. These results are in agreement with Poma, A. et al. [18] who found physiological and pathological conditions, of COVID-19, may affect the secretion of pituitary hormones. In a few cases, the infection was tracked down in cells of the adeno-and neurohypophysis [19].

Conclusion

We found that the pathophysiology of PCOS may be associated with the endothelial dysfunction and associated with corona virus infection. And the increased activity of the RAS in PCOS patients may be an indicator of hypertension and cardiovascular diseases, and therefore it can be used as a non-dependent indicator of insulin resistance to diagnose PCOS.

Acknowledgment

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not mine/ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- The author has signed an animal welfare statement.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad.

Author's Contribution Statement

S. E. A. performed the acquisition of data, analysis, interpretation, and drafting the manuscript. While F. M. K. did the analysis, design of interpretations, revision, and proofreading of the manuscript. F. E. A. diagnosed PCOS patients.

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تقييم نشاط أنزيم الرينين وأنزيم الكلوتاثيون اس ترانسفيريز في مصل سيدات يعانين من متلازمة تكيس المبايض وفايروس كوفيد -19 للتنبؤ بخطر الإصابة بأمراض القلب

شيماء عماد علي و فيحاء م. خليل

قسم الكيمياء ، كلية العلوم للبنات ، جامعة بغداد ، بغداد ، العراق.

الخلاصة

تؤثر جائحة الفيروس التاجي بشكل كبيرعلى الصحة العقلية والبدنية للمرأة. ان النساء المصابات بمتلازمة تكيس المبايض لديهن ميل كبير للعديد من عوامل الخطر المتعلقة بأمراض القلب والتمثيل الغذائي التي تزيد من فرصة حدوث المضاعفات الشديدة لـ فايروس كوفيد-19 وتظهر أيضًا احتمالية متزايدة لضعف الخصوبة. تتضمن هذه الدراسة معرفة مدى تأثير فايروس كوفيد-19 على مستويات الرينين ونشاط إنزيم الجلوتاثيون-إس-ترانسفيراز والمعايير البيوكيميائية الأخرى في النساء المصابات بمتلازمة تكيس المبايض . تشمل الدراسة 120 عينة من النساء تضم 80 سيدة مصابة بمتلازمة تكيس المبايض ، و 40 سيدة بصحة جيدة. تم تقسيم المجموعتين الرئيسيتين إلى مجموعات فرعية بناءً على ما إذا كانوا مصابين او غير مصابين بـ فايروس كوفيد-19. تم جمع عينات دم المصابين بمتلازمة تكيس المبايض في مستشفى كمال السامرائي في الفترة ما بين كانون الأول (ديسمبر) لغاية حزيران (يونيو) . تم قياس بعض المعايير البيوكيميائية لجميع مجموعات الدراسة والتي تشمل: - تحديد مستويات الرينين في مصل الدم باستخدام تقنية ELISA ، ونشاط GST ، ومستوى الدهون ، تم تقييم مستويات سكر الصيام بالطريقة الانزيمية ، وتم تقييم الهرمونات باستخدام جهاز VIDAS. قد تكون هناك علاقة بين مستويات السكر والرينين في متلازمة تكيس المبايض (متلازمة-شتاين- ليفينثال) ، بينما ارتبطت مستويات إنزيم مضادات الأكسدة (كلوتاثيون-اس-ترانسفيريز) عكسياً مع الوزن في متلازمة تكيس المبايض عند النساء حيث وجد أن مستويات الرينين كانت أعلى في النساء المصابات بمتلازمة تكيس المبايض مقارنة بالإناث الاصحاء. لكن أظهر مرضى متلازمة تكيس المبايض المصابين بفيروس كورونا انخفاضاً واضحاً في مستوى انزيم الرنين وانزيم الجلوتاثيون- اس- ترانسفيريز أكثر مما هو عليه في مرضى متلازمة تكيس المبايض غير مصابين بعدوى كورونا. البيانات الإحصائية اظهرت ان مستوى الرينين يرتبط سلبًا بنشاط الجلوتاثيون-إس-ترانسفيريز في حالة متلازمة تكيس المبايض . كانت مستويات الرينين أعلى في السيدات المصابات بمتلازمة تكيس المبايض الذي قد يؤدي إلى زيادة خلل في وظائف الكلى ويمكن توقع الإصابة بأمراض القلب والأوعية الدموية لدى مرضى متلازمة تكيس المبايض. قد يكون الانخفاض في قدرة مضادات الأكسدة ربما بسبب ارتفاع عدد الجذور الحرة التي يسببها الفيروس عند دخوله الجسم وارتفاع مستويات الرينين مما يؤدي إلى زيادة خطر الإصابة بمضاعفات متلازمة تكيس المبايض مثل أمراض القلب والأوعية الدموية.

الكلمات المفتاحية: الإنزيم المحول للأنجيوتنسين ، مؤشر كتلة الجسم ، فيروس كورونا 2019 ، الجلوتاثيون-اس-ترانسفيريز، متلازمة تكيس المبايض.