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Evaluation of Renin Level and Some Hormones in Patients with Polycystic Ovarian Syndrome

, Shereen F. Shaker[1], and Firas Shawqi Algburi[2]

[1] Department of Chemistry, College of Science, Tikrit University
[2] Department of Chemistry and Biochemistry, College of Medicine, Tikrit University

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Corresponding Author*:
Bayan N. Darweesh

ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is one of the most frequent gynecological endocrinopathy that occurs in premenopausal females. This study aimed to investigate the relationship between PCOS and Renin Angiotensin Aldosteron system, by measuring the levels of renin, ACE, Kallikren5, FSH and LH in follicular and luteal phase, in addition to evaluating the level of kallikrein 5, which belongs to the kallikrein-kinin system associated with the renin system. The renin-angiotensin system (RAS) is well known as regulator of electrolytes and blood pressure. Renin is previously considered a major enzyme in this system. The study included 90 blood samples for married women of childbearing age between (18-45) years. These samples included 70 samples for women with polycystic ovary syndrome, which were divided into two groups (35 samples in each one), the first was withdrawn in the follicular phase, named PA, and the second in the luteal phase of the menstrual cycle named PB. As for the remaining 20 samples, they represented the control group of healthy women, which were also divided into two groups (CA and CB) as happened with the patient group. In this study, the levels of serum Renin, ACE, LH, FSH and Kallikren5 were evaluated in PCOS patients and compared with the control group in both phases. The results were analyzed by T-Test and ROC. There were no significant differences between patients and the control group in terms of ACE activity or FSH ($P>0.05$). The results demonstrated that LH, Renin, and Kallikren5 were higher in patient group in both phases ($P<0.05$). Renin Angiotensin system was affected by PCOS.

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تقييم مستوى الرينين وبعض الهرمونات لدى مريضات متلازمة المبيض متعدد الأكياس

بيان نعمت درويش[1], شيرين فاروق شاكر[1]، فراس شوقي الجبوري[2]

[1]قسم الكيمياء، كلية العلوم، جامعة تكريت

[2]فرع الكيمياء والكيمياء الحياتية، كلية الطب، جامعة تكريت

الملخص

تعد متلازمة المبايض متعددة الأكياس (PCOS) واحدة من أكثر حالات اعتلال الغدد الصماء النسائية شيوعًا التي تحدث عند الإناث قبل انقطاع الطمث، وقد أجريت هذه الدراسة للتحقق من العلاقة بين متلازمة تكيس المبايض ونظام الرينين أنجيوتنسين ألدوستيرون، عن طريق قياس مستويات الرينين ، الإنزيم المحول للأنجيوتنسين ، كاليكرين 5 ، FSH و LH ، في المرحلة الجريبية والأصفرية، بالاضافة الى تقييم مستوى انزيم الكاليكرين5 الذي ينتمي الى نظام الكاليكرين كاينين المرتبط بنظام الرينين. نظام الرينين أنجيوتنسين(RAS) معروف جيدًا كمنظم للالكتروليتات وضغط الدم. يعتبر الرينين إنزيمًا رئيسيًا في هذا النظام. اشتملت الدراسة على 90 عينة دم للسيدات المتزوجات في سن الإنجاب بين (18-45) سنة، منها 70 عينة للنساء المصابات بمتلازمة تكيس المبايض، تم تقسيمها إلى مجموعتين (35 عينة في كل واحدة)، تم سحب الأولى في المرحلة الجرابية، تسمى PA، والثانية في المرحلة الأصفرية من الدورة الشهرية تسمى PB، مقارنة بالمجموعة الضابطة، والتي تضمنت 20 عينة من النساء الأصحاء، والتي تم تقسيمها إلى مجموعتين (CA و CB) أيضًا ، كما في مجموعة المرضى. في هذه الدراسة، قمنا بتقييم مستويات الرينين في المصل، ACE، LH، FSH و Kallikren5 في مريضات متلازمة تكيس المبايض ومقارنتها مع المجموعة الضابطة في كل من مرحلتي الجريب والأصفر، تم تحليل النتائج بواسطة T.Test و ROC ، ولم تكن هناك فروق ملحوظة بين المرضى والضوابط من حيث نشاط ACE أو FSH.أظهرت النتائج أن LH و Renin و Kallikren5 كانت أعلى في مجموعة المرضى في كلتا المرحلتين (P <0.05). يتأثر نظام الرينين أنجيوتنسين بمتلازمة المبيض متعدد الأكياس.

الكلمات المفتاحية: متلازمة المبيض متعدد الاكياس، الهورمون اللوتيني، الهورمون المنشط للحوصلة، الانزيم المحول للانجيوتينسين، الرينين

Introduction

The majority of women of childbearing age are affected by this endocrine condition of PCOS, which is characterized by polycystic ovaries, hyperandrogenism, insulin resistance, and persistent anovulation. A more reliable, evidence-based international guideline for the evaluation and management of PCOS has been released in 2018 as opposed to the Rotterdam Diagnostic Criteria issued in 2013 [1]. The etiology of the syndrome includes a combination of genetic and environmental factors [2].

The Rotterdam Criteria for diagnosing PCOS in adults suggested two of the following three additional diagnostic criteria: lack of ovulation, clinical and/or biochemical symptoms of hyperandrogenism, and polycystic ovarian morphology as determined by ultrasound [3].

The PCOS disease is also accompanied by a lack of progesterone secretion in the second half of the menstrual cycle, which is the hormone specialized in removing the lining of the uterus and its descent, known as the menstrual cycle or menstruation [4]. The metabolic defect characterized by insulin resistance is so common in PCOS that it can be considered an integral part of the syndrome. Insulin resistance results in compensatory hyperinsulinemia, which promotes luteinizing hormone release and raises ovarian androgen synthesis. Dyslipidemia is a side effect of insulin resistance that also increases the risk of type 2 diabetes and cardiovascular disease in PCOS-affected women [5].

PCOS is associated with psychological problems such as anxiety, depression, and body image disturbances [6].

Insulin resistance and the risk of developing diabetes in this syndrome are closely related to obesity. PCOS is rarely diagnosed in females of normal weight, as there are many symptoms of metabolic syndrome, especially hyperinsulinemia, that differ from one patient to another. It is believed that women with PCOS have a 30-50% pregnancy loss rate in the first trimester [7]. Insulin resistance is also affected by the renin-angiotensin-aldosterone system [8]. The symptoms include: stopping of menstruation (at childbearing age); absence or lack of ovulation, with or without hemorrhagic monthly ovulation acne; hirsutism; hair loss or thinning of the scalp [9]; presence of more than 12 ovarian cysts [10]; weakness and a disturbance in the ovulation process, which leads to a delay in pregnancy; feeling of sudden mild or sharp pain, which is usually in the lower abdomen, occurring during or shortly after the beginning of menstruation or at its end; weight gain, usually in the torso and limbs, happening due to a disorder in the body fat level [4]. There are several theories with a lot of evidence indicating a genetic link that causes the appearance of PCOS in groups of women, and the presence of the

syndrome in some individuals before the first menstrual period [6]. Both obese and weak women with the syndrome show insulin resistance [11].

Renin Angiotensin Aldosteron System (RAAS)

Blood volume and systemic vascular resistance are both controlled by RAAS. Low arterial pressure causes short-term responses from autonomic baroreceptors, while larger long-term changes are caused by RAAS [12]. This system consists of proteins, peptides, enzymes, and receptors. It has been characterized in diverse organs and systems, including the brain, trophoblast, pancreas, adipose tissue, and gonads [13]. Renin, angiotensin, and aldosterone are the three primary chemical substances that make up this system. These three substances reduce salt transport to the distal convoluted tubule and increase arterial pressure in response to renal hypotension. So, the body through these mechanisms can raise blood pressure in a prolonged manner [12].

Major components of RAAS have been shown to be present in the ovary including renin and prorenin, angiotensinogen, ACE, angiotensin Ang II and its receptors AT1 and AT2, as well as those involved in reproductive processes such as folliculogenesis, steroidogenesis, and maturation, ovary and oocytes [14]. Kallikren5 is a member of the human kallikrein gene family of serine proteases. KLK5 has been shown to regulate estrogen/progestin hormones. It is highly expressed in hormone-responsive or endocrine tissues such as testis, ovary, breast, and skin [15]. Several KLK genes are transcriptionally regulated by steroid hormones and differentially expressed in hormone-related malignancies [16].

Based on the above, this study aims to evaluate the relationship between RAAS and PCOS, in addition to evaluating the level of some sex hormones and determining the difference in the level of RAAS enzymes and sex hormones in the follicular and luteal phases of the menstrual cycle.

Subject and Method

This study was conducted in the laboratories of Samarra General Hospital from mid-September to the end of December 2022. It included (90) blood serum samples of married women of childbearing age, ranging in age from (18-45) years. These samples were divided into four groups, as follows: Control group CA: Ten blood samples were drawn from healthy women during the follicular phase of the menstrual cycle. Control group CB: Ten blood samples were drawn from healthy women during the luteal phase of the menstrual cycle. Patient group PA: 35 blood samples were drawn from PCOS women during the follicular phase. Patient group PB: 35 blood samples were drawn from PCOS women during the luteal phase. Inclusion criteria: married women of age ranging between (18-45) years, with PCOS, without tumors in reproductive system, urinary tract, adrenal and pituitary gland. Exclusion criteria: women who have cancer of the reproductive system, urinary, or pituitary and adrenal glands, and those taking antihypertensive medication.

Estimation of FSH and LH Levels in Serum

Enzyme linked immunosorbent assay (ELISA) kits were used to evaluate LH and FSH levels (The HUMAN FSH and LH kits).

Estimation of ACE, Renine, Kallikern5 Levels in Serum

The working principle of this kit is based on the ELISA technique (Fine Test ACE, Renine, and Kallikren5 kits).

Statistical Analysis

In this regard, SPSS22 program was used to analyze the results, by extracting the mean and standard deviation. In addition, T-test was used to analyze the differences between the main and secondary groups. The significant differences were chosen for those groups under the probability level ($p \leq 0.05$). The Receiver Operating Characteristic Curve (ROC) test was also used to determine the accuracy of the diagnosis.

Results and Discussion

Table 1 shows the mean and standard deviation for the variables (renin, ACE, LH, FSH, Kallikren5) and the P-values.

Table 1. Serum level of LH, FSH, ACE, Renine and KLK5 in studied groups

Parameters	Mean±SD			
	CA(n=10)	**CB(n=10)**	**PA(n=35)**	**PB(n=35)**
Renin (Pg/ml)	**163.1±50.8**	**240.2±118.3**	**396.6±147.2**	**852.9±279.9**
ACE (ng/ml)	**38.54±8.55**	**41.78±10.4**	**47.51±15.5**	**47.37±10.9**
Kallikren5(ng/ml)	**0.73±0.24**	**0.77±0.21**	**0.5±0.25**	**0.56±0.26**
LH (IU/L)	**5.99±1.24**	**7.38±1.14**	**7.95±2.79**	**9.72±2.25**
FSH (IU/L)	**5.37±1.8**	**4.15±1.32**	**4.59±1.41**	**4.41±2.17**
	P-value (T-Test)			
	CA/CB	**CA/PA**	**CB/PB**	**PA/PB**
ACE	**0.46**	**0.12**	**0.103**	**0.96**
Kallikren5	**0.71**	**0.01****	**0.02***	**0.4**
Renin	**0.04***	**<0.001****	**<0.001****	**<0.001****
LH	**0.04***	**0.003****	**0.003****	**0.005****
FSH	**0.17**	**0.15**	**0.6**	**0.65**

The results showed a significant increase in the level of luteinizing hormone in comparison between the control group (CA 5.99 ± 1.24) and the infected group (PA 7.95 ± 2.79) ($P < 0.05$). A significant increase was observed between the control group (CB 7.38 ± 1.14) and the infected group (PB 9.72 ± 2.25) for the same hormone. This rise may be attributed to PCOS, which prevents the ovaries from producing hormones in the proper ratios. The pituitary gland detects this dysfunction and responds to it due to the feedback by secreting aberrant levels of LH, a hormone required for ovulation and ovarian maturation. Ovaries are functioning normally and producing eggs, while a high quantity of LH secretion signals ovarian failure or the early stages of menopause in woman [17]. These results agreed with those found in [18,19]. The rise of the hormone in the luteal phase is consistent with one of the previous studies [20]. This study also showed that there was no significant difference in the level of FSH in comparison between CA (5.37 ± 1.8) and PA (4.59 ± 1.41), as well as between CB (4.15 ± 1.32) and PB (4.41 ± 2.17) ($P > 0.05$). These results are consistent with those found in [21], while they are contrary to another study [22].

The results showed that there was no significant difference in the level of ACE enzyme when comparing between CA (38.54 ± 8.55) and PA (47.51 ± 15.5), as well as when comparing between PA (47.51 ± 15.5) and PB (47.37 ± 10.9) and between CB (41.78 ± 10.4) and PB (47.37 ± 10.9) ($P > 0.05$). This result is consistent with another study, proving that there was no significant difference between the enzyme levels of those with PCOS and the control group in the follicular phase [23]. While this result contradicts another study, showing that the significant increase in the level of ACE may be due to the positive correlation between the level of the enzyme and insulin resistance and between the enzyme and the level of LH [24].

The results of the study indicated a highly significant increase in the level of renin ($P<0.01$) when comparing the groups CA (163.1 ± 50.8) and PA (396.6 ± 147.2), as well as between CB (240.2 ± 118.3) and PB (852.9 ± 279.9). This result is consistent with a previous study, showing that high levels of renin in blood serum reflect the activity of the renin-angiotensin system (RAS), and may reflect the increased number of follicles that contain

a high concentration of angiotensin II receptors [25]. There was a significant difference between CA and CB ($P < 0.05$). This result agrees with the study that shows a higher level of renin in the luteal phase than in the follicular phase, and an increase in urinary prorenin and aldosterone. Thus, it seems likely that there is a relationship between progesterone and an increase in prorenin [26].

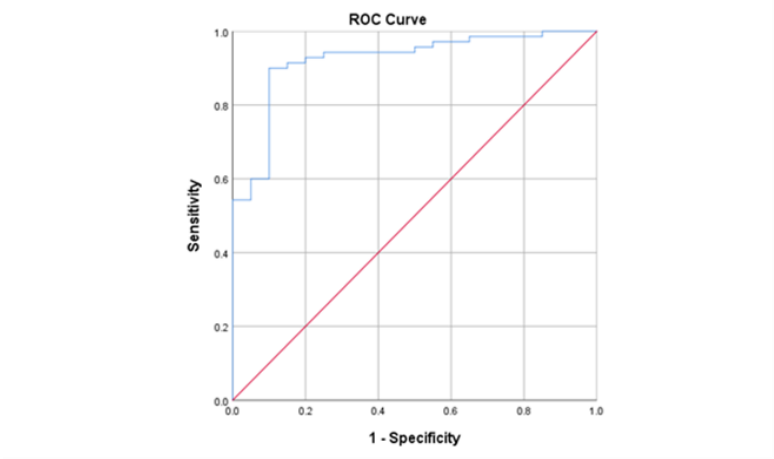
As for kallikrein 5, the results showed a significant difference when comparing between CA (0.73 ± 0.24) and PA (0.5 ± 0.25)($P< 0.05$), PB(0.56 ± 0.26) and CB (0.77 ± 0.21). Kallikrein is associated with the level of renin, as kallikreins have been identified in tissues as one of the activators that convert prorenin to renin. It is shown that plasma kallikrein is the main factor for activating prorenin. ANG II receptors are involved in cross-talk between the renin-angiotensin system (RAS) and the kallikrein-kinin (KKS) system. The communication between ANG II and its receptors appears to involve many interactions between KKS and RAS. This fine-tuning of these systems helps to maintain the homeostasis of blood pressure regulation and other biological processes. RAS stimulates renal BK bradykinin production and cGMP formation through ANG II receptor 2 (AT2). Renin, but not the angiotensin I receptor, reduces renal BK levels during salt depletion [27].

ROC was used to evaluate the performance of chemical tests in the biochemical diagnosis of the disease in this study, and to determine the appropriate cut-off values for the different variables. Sensitivity and specificity were also calculated. The area under the ROC curve (AUC) was used as an abbreviated measure of diagnostic accuracy. Kallikren5 and FSH failed to diagnose the PCOS, because the AUC value was too low, whereas Renine and LH were useful in diagnosing PCOS in an acceptable rate.

Table 2. Receiver Operating Characteristic Curve (ROC)

Parameters	Sensitivity	Specificity	CUT OFF	AUC
Renin	90%	90%	273.5	0.922
ACE	%69	80%	41.19	0.708
Kallikrein5	1.4%	100%	1.204	0.245
LH	54%	100%	9.15	0.751
FSH	5.7%	100%	8.16	0.427

Through ROC statistical analysis, sensitivity, specificity, and cut-off values were found for each variable. As shown in table (2), Kallikrein 5 showed the lowest level of sensitivity and the highest level of specificity, which means that it has no diagnostic significance in PCOS, while renin showed the highest level of sensitivity and specificity among the other parameters.



Figurer 1. ROC of Renin: sensitivity and specificity of PCOS patients and controls

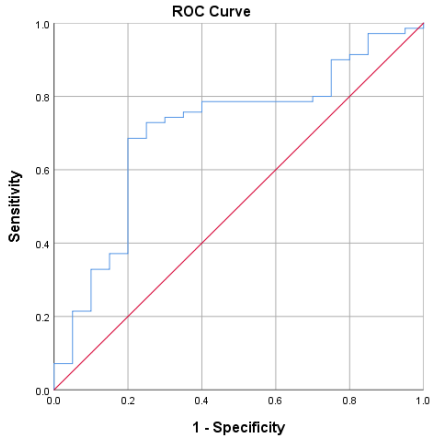


Figure 2. ROC of ACE: sensitivity and specificity of PCOS patients and controls

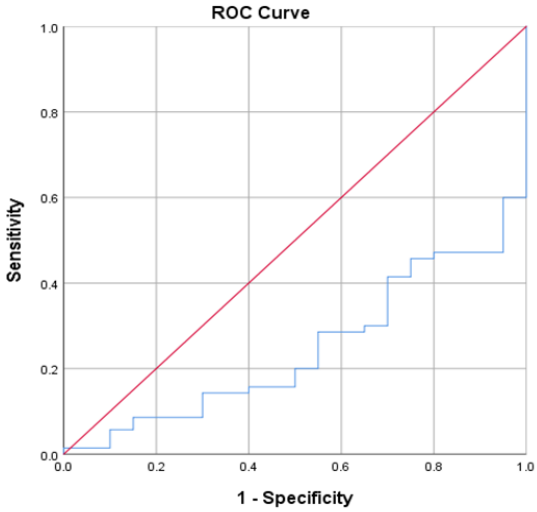


Figure 3. ROC of Kallikren5: sensitivity and specificity of PCOS patients and controls

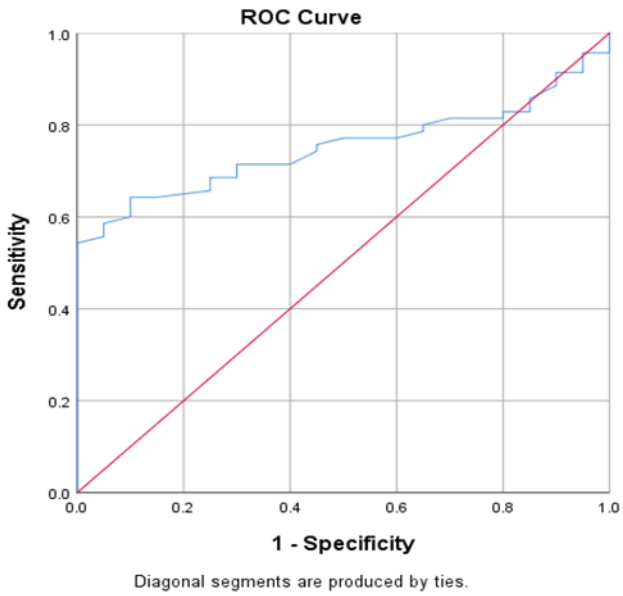


Figure 4. ROC of LH: sensitivity and specificity of PCOS patients and controls

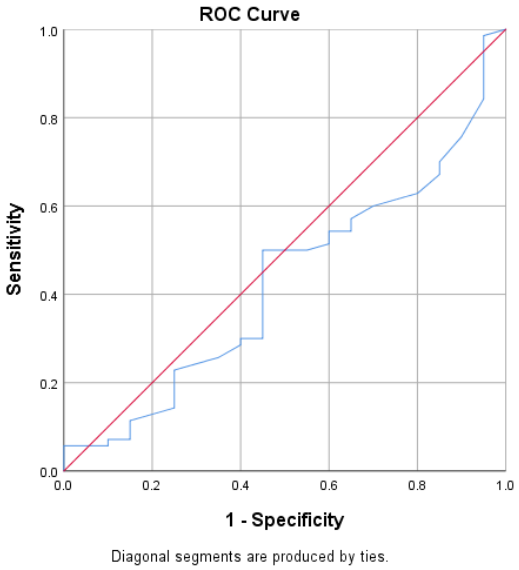


Figure 5. ROC of FSH: sensitivity and specificity of PCOS patients and controls

Conclusion

PCOS affects the sex hormones and thus it affects women's fertility and childbearing. PCOS leads to an imbalance in blood pressure and the level of electrolytes in the blood.

Recommendations

This study recommends following a healthy diet to reduce the symptoms of PCOS, and medical instructions to maintain the level of sex hormones, as well as monitoring blood pressure and the level of electrolytes to know the activity of the renin-angiotensin-aldosterone system.

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